The effects of male philopatry on reproductive behaviors and fitness in Columbian ground squirrels (*Urocitellus columbianus*)

by

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Abstract

Decreased fitness is a consequence of inbreeding in many species. As a result, a variety of behavioral mechanisms, such as dispersal, are employed be mammals to avoid mating with relatives. Columbian ground squirrels (*Urocitellus columbianus*) are a polygynandrous species in which females remain philopatric, and males have a mixed dispersal pattern, with some males emigrating and others remaining philopatric residents. The goals of this study were to determine the effects of the presence of close female kin (mothers and littermate sisters) on the mating habits of Columbian ground squirrels, explain the mixed dispersal pattern in males, and to determine whether inbreeding occurs in this species. We additionally compared the fitness (measured as number of mating partners and offspring produced) and several other variables (mass, hibernation emergence date, age, and length of time living in the population) of males to detect whether residency status (immigrant compared to philopatric resident) influences fitness, and, if so, what variables are related to the difference. We addressed these goals using a longitudinal dataset of the reproductive behaviors of a population of Columbian ground squirrels in Sheep River Provincial Park, Alberta, Canada. Our analysis included data from 2005-2015 and had a total sample of 56 individual adult males and 90 individual adult females. Some individuals lived in the population multiple years, resulting in 127 samples of males and 244 samples of females (191 of which had logs of mating partners). Through our analysis, we found that the presence of close female relatives resulted in decreased male mating likelihood; male samples that did not co-occur with their mother were 1.34 times more likely to mate with one or more females, and male samples that did not co-occur with littermate sister(s) were 1.45 times more likely to mate. The data supports that the decreased mating likelihood may be a result of age. In addition, although male samples and female samples co-occurred equally with their mothers, females were 1.86 times more likely to co-occur with another littermate sister. This may be a result of sex-biased aggression from mothers towards sons. In regards to inbreeding, pairs of a non-related male and females mated 8.83 times more often than related pairs, and only one instance of inbreeding between a male and close female relative (mother) were detected during the study. Thus, inbreeding is avoided in this species, potentially through a combination of dispersal and recognition of relatives. By comparing resident male samples and immigrant male samples, we found that resident male samples were 1.28 times more likely to mate than immigrant male samples, and resident male samples had significantly more mating partners on average (7.4±5.1 SD mates compared to 4.7±5.1 SD mates; p=0.008). We concluded that this was the result of the effects of age and emergence date differences between resident male and immigrant male samples. Interestingly, despite more mating partners, there was no significant difference in the average number of offspring produced by resident and immigrant male samples (6.1±6.0 SD offspring and 5.0±6.8 SD offspring; p=0.348). This may be a result of inbreeding depression from distant relatives mating or moderate inbreeding. Immigrant male samples had a nearly significant trend of producing more offspring per mate compared to resident male samples (interaction between residency status and number of mates in regards to number of offspring produced: p=0.092). For adult male resident samples, for every one mate increase there was a 0.9 offspring increase in number of offspring produced (r^2=0.50, p<0.001), but for adult immigrant male samples, for every one mate increase there was a 1.1 offspring increase in number of offspring produced (r^2=0.69, p<0.001).

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Chapter I

The effects of co-occurrence with close female relatives on mating behaviors of male Columbian ground squirrels

Abstract

Inbreeding can cause detrimental fitness consequences in many species. Dispersal from a natal population, particularly dispersal that is sex-biased or occurs prior to reproductive maturity, is one behavioral mechanism to avoid inbreeding. The goal of our study was to determine the effects of the presence of close female kin on the mating habits of a polygynandrous species, Columbian ground squirrels, and to determine whether inbreeding occurs in this species. We proposed a series of hypotheses to explain the male dispersal pattern present in Columbian ground squirrels, in which some males remain philopatric and others emigrate, and to determine and explain any inbreeding that occurs in this species: 1) philopatric males remain in the population, and don't emigrate, because they have no surviving, closely related, female relatives; 2) philopatric males have close female relatives present, but they recognize their presence and avoid inbreeding by not mating with any females; 3) philopatric males mate even when occurring with close female relatives, but they avoid inbreeding by recognizing, and not mating with, close female relatives; 4) philopatric males mate with close female relatives, but they do not produce any offspring; 5) philopatric males mate with close female relatives and produce offspring with success levels similar to that of immigrant males. We found that males and females co-occur with their mothers with similar likelihood, but that females are 1.86 times more likely to co-occur with littermate sisters. Males without co-occurring mothers were 1.34 times more likely to

mate with one or more females compared to males without co-occurring mothers, and males without co-occurring littermate sisters were 1.45 times more likely to mate. Non-related male-female pairs (i.e. not a son-mother or brother-littermate sister pair) mated 8.83 times more often (700 out of 2200 potential pairs) than related pairs (1 out of 28 potential pairs). We suggest that the sex-bias in co-occurrence with littermate sisters may be related to female aggression directed at sons, based on previous research regarding interactions between sciurid mothers and offspring. The decreased likelihood of males mating when co-occurring with their mothers may be due to age. Males that did not co-occur with their mothers were significantly older than those that did, and older males are more likely to mate. Ultimately, despite the high potential for inbreeding in this species, only one instance, between a mother and son, was observed over the 11-year study, and this instance occurred during an unusual year when only two adult males mated, compared to average of 8.2±3.8 SD males mating each year. Columbian ground squirrels are able to recognize mothers and littermate siblings, which could potentially be related to the low levels of inbreeding. In the one instance of inbreeding, the mother produced offspring, but none were fathered by her son. To further determine if males, females, or both are preventing inbreeding in this species, we recommend that future studies address mother aggression towards offspring and address whether males, females, neither, or both attempt to mate with close relatives but are rejected.

Introduction

Inbreeding avoidance

Inbreeding, or mating between close relatives, and the subsequent potential for inbreeding depression, or reduced fitness of the offspring of these inbred pairs, have been topics of interest amongst the scientific community for many years (Hendrick and Kalinowski, 2000; Pusey and Wolf, 1996; Lawson Handley and Perrin, 2007). Much of the research on these topics has addressed the consequences of inbreeding. Of equal interest, however, are the studies on the mechanisms and tactics animals use to avoid and prevent inbreeding. To understand why research on inbreeding avoidance is necessary, one much first become familiar with the detrimental effects of inbreeding.

Through inbreeding, genetic variation is lost (Allendorf and Leary, 1986). Because fewer new genetic combinations are created between inbred pairs, if inbreeding is prevalent in a population, deleterious alleles may become fixed, with the resulting disadvantageous phenotype consistently expressed in all individuals (Pusey and Wolf, 1996; Allendorf and Leary, 1986). In Pusey and Wolf's (1996) meta-analysis of inbreeding, loss of genetic variation was shown to cause sperm deformities, sterility, and decreased courtship frequency. The researchers also found that, in a variety of experimental pairing studies of rodents, paired littermates had reduced reproductive rates compared to non-littermate pairs, and paired siblings were less likely to produce litters than paired cousins or non-relatives. Allendorf and Leary (1986) had similar findings through their meta-analysis, in which they concluded that loss of genetic variation can not only affect development, survival, and growth rate of individuals, but it can also make entire populations more susceptible to diseases and parasites.

Despite the potential for the issues above, it's noteworthy that inbreeding depression is not universal among species. For example in the yellow-bellied marmot, although inbred offspring had decreased survival, males that inbred had the same reproductive success as those that didn't inbreed (Olson et al., 2012). In the dwarf mongoose, breeding pairs are commonly related, but close inbreeding has no effect on offspring production or adult survival of inbred individuals (Keane et al., 1996). These examples are exceptions, however.

As a result of the detrimental effects of inbreeding depression, evolution has favored the development of mechanisms to prevent and avoid inbreeding. These mechanisms include: (1) recognition of relatives (Hoogland, 1992; Dobson et al., 1997; Waser et al., 2012; Michener and Michener, 1973; Harris and Murie, 1982; King, 1989; Leclaire et al., 2012, Mateo, 2006), (2) attraction to genetic dissimilarity (Mays and Hill, 2004; Van Bergen et al., 2013), (3) mating with multiple individuals (polygyny, polyandry, and extra-pair mating) (Birkhead, 2010; Zeh and Zeh, 2001; Murie, 1995; Winters and Waser, 2003; Waser and DeWoody, 2006), (4) cryptic female choice (Birkhead, 1998; Birkhead, 2010; Holt, 2011), (5) single sex dispersal (Pusey, 1987; Greenwood, 1980; Dobson, 1982; Holekamp, 1984a; Lawson Handley and Perrin, 2007) and (6) dispersal prior to sexual maturity (Greenwood, 1980; Holekamp, 1984a; Lawson Handley and Perrin, 2007) . Each of these will be discussed below.

Many sciurid and small mammal species can recognize relatives, sometimes through methods such as scent or social learning. Black-tailed prairies dogs avoid mating with close kin, such as parents, offspring, and full and half siblings, but they regularly breed with more distant relatives (Hoogland, 1992). Females refuse to mate with individuals within their breeding groups (coteries) but mate randomly within their subpopulation (colony) (Hoogland, 1992; Dobson et al., 1997). Individuals in adjacent coteries in the colony are sometimes close kin as well, however, which supports that individuals recognize their kin through social learning rather than scent (Dobson et al., 1997). Banner-tailed kangaroo rats also use social cues to recognize relatives (Waser et al., 2012). Because both sexes of this species are highly philopatric, they have increased chances of inbreeding. Females, however, have the ability to discriminate kin through the familiarity they develop early in life.

Scent recognition occurs in Richardson's ground squirrels, Columbian ground squirrels, meerkats, and Belding's ground squirrels. Lab experiments by Michener and Michener (1973) found that yearling and adult Richardson's ground squirrels were rapidly able to recognize other individuals as familiar or unfamiliar following the seven to eight-month hibernation period. Recognition involved nasal contact. Through scent cube experiments, Columbian ground squirrels were able to distinguish between strange, neighboring, and resident males (Harris and Murie, 1982). Both males and females sniffed cubes of oral gland scent from other (strange and neighboring) males longer than cubes with resident male scent, and they sniffed cubes with strange male scent longer than those with neighboring male scent. Researchers hypothesized that responsiveness to scent decreased as familiarity of the scent increased. Although there was no support that adult could recognize individual identity, in addition to familiarity, researchers suggested that it was possible. A later study, however, determined that females sharing natal burrows do indeed act as though they recognize one another (King, 1989). Through behavioral observations, agonistic interactions were lower among uterine kin than non-uterine kin, and non-littermate sisters had a higher proportion of agonistic encounters than did uterine kin.

Scent experiments with meerkats have resulted in conclusions similar to those of experiments with Columbian ground squirrels (Leclaire et al., 2012; Harris and Murie, 1982). When dominant females were presented with anal gland secretions from unfamiliar males of varying levels of relatedness, they spent more time smelling scents of related individuals and were more vigilant. Although this result is opposite that of Columbian ground squirrel scent experiments (Harris and Murie, 1982), females alternatively may use a scent, phenotype matching mechanism, using their own scent or the scent of known-kin as comparisons, to discriminate unknown kin from non-kin. Scent cube experiments with Belding's ground squirrels yielded results more similar to those of Columbian ground squirrels (Mateo, 2006; Harris and Murie, 1982). Yearling and juvenile Belding's ground squirrels spent more time sniffing scent cubes with scents of unfamiliar individuals than familiar individuals. Mateo (2006) hypothesized that the major histocompatibility complex (MHC) may be related to scent recognition. Due to the high number of genes involved in the MHC, the likelihood of any two individuals having the exact same MHC haplotype is low, but because relatives share so many genes, the MHC by-products may act as an indicator of relatedness (Beynon and Hurst, 2004; Mateo, 2006). The scents used in Mateo's (2006) experiment came from several sources on the body (oral, anal, dorsal, pedal, and supraorbital glands), and the interactions between the by-products of the MHC and the bacteria on the gland surfaces or gastrointestinal tract creates unique, recognizable odors for each individual. Another signal of scent recognition occurs in rodents through pheromone bound proteins in urine, known as major urinary proteins (MUPs) and alpha 2u globulins (A2Us) (Beynon and Hurst, 2004). These proteins are products of several discrete genes and, similar to MHC byproducts, a wide variety of haplotypes exist, thus MUPs may also act as a signal of relatedness.

Another, less studied, method of inbreeding prevention is attraction towards genetically dissimilar individuals. A meta-analysis found that several studies have supported that females are attracted towards, and have post-copulatory choice towards, genetically dissimilar mates (Mays and Hill, 2004). In some species, this choice seems to be based on scents and chemicals, particularly those related to MHC diversity of individuals, in which females are attracted towards males with a dissimilar MHC haplotype. These findings are particularly interesting in light of the scent recognition abilities in Belding's ground squirrels, which was hypothesized to be related to MHC (Mateo, 2006). In a more concrete example, Wedekind et al. (1995) supported through experimental studies that female humans are attracted to males with dissimilar MHC haplotypes. In a unique study, individuals in a butterfly species (*Bicyclus anynana*) were found to be able to recognize inbred individuals through scent (Van Bergen et al., 2013). Inbred males of the species produce significantly less sex pheromone, and they also have significantly reduced mating success. Although research on genetic dissimilarity is less prevalent, the mechanism provides another pre-mating technique of avoiding inbreeding.

In the event that individuals are not able to avoid mating with potentially close relatives, mating with multiple individuals may mitigate the negative effects of inbreeding. For instance, females can gain genetic benefits from copulating with multiple males to compensate for a particular partner's infertility (Birkhead, 2010). A meta-analysis of polyandry provided several hypotheses regarding genetic benefits females may gain from mating with multiple males (Zeh and Zeh, 2001). These hypotheses included: 1) extra-pair copulations compensate for poor quality males; 2) bet-hedging hypothesis, in which polyandry enables females to guard against mate choice errors; 3) offspring diversity hypothesis, in which multiple mates increase genetic variability of offspring, which in turn enhances a mother's fitness by buffering at least some offspring against environmental uncertainty that could wipe out individuals with certain genetic combinations; and 4) genetic compatibility avoidance hypothesis, in which polyandry provides females with the opportunity to use post-copulatory mechanisms to decrease the chances of fertilization by genetically incompatible sperm.

Studies on several sciurid and small mammal species have also addressed the topic of multiple mating. Columbian ground squirrel females mate with multiple males, which promotes good genes through sperm competition (Murie, 1995). The trend for this species is that the number and proportion of juveniles surviving to yearling age increases as the number of mates of the mother increases. In banner-tailed kangaroo rats, polyandry is believed to help reduce the relatedness of mates (Winters and Waser, 2003). Females of the species tend to be closely related to their nearest neighbor, but despite this, many litters have multiple paternity and high genetic heterozygosity, and non-nearest neighbors were found to have sired three-quarters of the offspring in one study (Winters and Waser, 2003; Waser and DeWoody, 2006). In years with high population density, offspring are less likely to be inbred, possibly because females have more mating options of unrelated males (Waser and DeWoody, 2006). Researchers have also suggested that the high paternity of non-nearest neighbors may be due to cryptic mate choice, natural abortion in utero due to inbreeding depression, or inbred offspring having more frequent early mortality rates (Winters and Waser, 2003; Waser and DeWoody, 2006). In meerkats, although extra-pair mates and extra-pair maternity occur, inbreeding depression has been noted in several traits, including mass at emergence and juvenile survival (Leclaire et al., 2013). In this species, groups consist of a dominant breeding pair and their offspring. Resident breeding males tend to be immigrants, but individuals only disperse a short distance, thus even immigrants have a high chance of being related to their breeding pair mate. This may be a possible explanation for the inbreeding depression. Additionally, although extra-pair mates were more heterozygous, extra-pair pups were of equivalent heterozygosity of within-pair pups.

A particularly controversial theory of inbreeding prevention is cryptic female choice, or the ability of females to “choose” the sperm of one male over another post-mating to avoid incompatible genetic combinations (Birkhead, 1998). One way to choose sperm is through ejecting sperm post-mating, a tactic that has been recorded in both domestic and feral fowl (Birkhead, 1998; Birkhead, 2010). A variety of sciurid species form copulatory plugs, potentially within 30 minutes of mating and, in Columbian ground squirrels, remaining for up to 19 hours post-mating (Murie and McLean, 1980). Columbian ground squirrels have been observed removing copulatory plugs post-mating (Raveh et al., 2011). These plugs prevent the likelihood of fertilization of future males in polyandrous species, like the Columbian ground squirrels, but by removing the plugs, females increase the likelihood of fertilization by multiple males (Murie and McLean, 1980; Raveh et al., 2011). If fertilization has already occurred, differential abortion of embryos could occur (Birkhead, 1998). This tactic is mostly theoretical because providing empirical support that females are selecting which embryos to abort is difficult. Less theoretical, however, is sperm storage. Certain reptile, fish, bird, and amphibian females are able to store sperm for months, and even years in some cases, in their reproductive tracts (Birkhead, 2010; Holt, 2011). Many mammals are capable of short term storage, up to seven days within the oviductal isthmus, but some bat species can actually store spermatozoa for several months in the uterus or oviduct, possibly in pockets in the epithelial cells (Holt, 2011).

A mechanism of inbreeding avoidance that has a considerable amount of supportive research evidence is single sex dispersal. If a single sex of a species disperses further than the other sex in social species, the chances of inbreeding are reduced. In birds, the dispersing sex is typically the females, whereas in mammals, typically males disperse (Pusey, 1987; Greenwood, 1980; Lawson Handley and Perrin, 2007). In mammals, females have the higher parental investment, and they benefit by having a familiar, stable location to raise their offspring; they have a better chance of achieving this if they remain philopatric (Greenwood, 1980; Pusey, 1987). Male mammals, however, are primarily concerned with increasing their fitness by mating with as many females as possible, and by dispersing, they increase their access to unrelated mates. Interestingly, a meta-analysis of mammals found that while juvenile males are the primary dispersers in most polygynous and promiscuous species of mammals, in monogamous mammal species, both juvenile males and females disperse relatively equally (Dobson, 1982). In most bird species, both males and females have equal investment in offspring, but because males compete for and defend territories to attract mates, they benefit more from consistently retaining or returning to a particular territory, similar to female mammals (Pusey, 1987). As a result, female birds benefit by dispersing to avoid inbreeding. Alternatively, some researchers argue that, in birds, there is no relationship between social mating system and sex-biased dispersal based on phylogenetic analyses (Mabry et al., 2013). Mabry et al. (2013) found in their meta-analysis that while 83% of socially-monogamous birds exhibited female-biased dispersal, 89% of non-monogamous bird species also exhibited it.

Ground-dwelling sciurids display the typical male-biased dispersal tendency of mammals (Holekamp, 1984a). In round-tailed ground squirrels (Dunford, 1977), California ground squirrels (Dobson, 1982), Belding’s ground squirrels (Holekamp, 1984b), Columbian ground squirrels (Festa-Blanchet and King, 1984; Wiggett and Boag, 1989; Neuhaus, 2006), and black-tailed prairie dogs (Dobson, et al., 1997), juvenile males are the primary dispersers. To describe a few examples in depth, in a study on Columbian ground squirrels, of the reproductively active individuals, 30% of males were immigrants, whereas only 1.8% of females were immigrants (Neuhaus, 2006). In the closely related Belding's ground squirrels, a study found that by the age of 55 weeks, all of the males had emigrated, whereas almost all of the females remained not only in the population, but also within the boundary of their mother's home range (Holekamp, 1984b). In a non-ground squirrel species, the Malagasy mongoose (a carnivorous small mammal) males are believed to disperse farther than females (Schneider et al., 2016). Schneider et al. (2016) determined this not only using observations, but primarily through haplotype analysis, in which males were found to have higher haplotype diversity and lower relatedness to population members. Genetic analysis is a less utilized method to determine dispersal patterns, but it provides a contrasting method to the potentially more error prone observational studies, particularly in rare species or species that are difficult to observe.

A noticeable trend amongst the examples above is that the dispersers are primarily juveniles. Dispersal prior to sexual maturity is another mechanism that minimizes inbreeding (Greenwood, 1980; Lawson Handley and Perrin, 2007). This tactic is particularly common in ground squirrels and ground-dwelling sciurids, such as California ground squirrels (Armitage, 1981; Dobson, 1979). In most ground-dwelling sciurids, natal dispersal occurs during their first summer, between one month after initial emergence and one month before hibernation (Holekamp, 1984a). Some species, however, delay until the spring of their juvenile year, while others delay even later until their second (yearling) summer or third summer as two-year-olds. In yellow-bellied marmots (Armitage and Downhower, 1974; Holekamp, 1984a) and Columbian ground squirrels (Wiggett and Boag, 1989; Festa-Blanchet and King 1984; Holekamp, 1984a), dispersal occurs primarily among yearlings. Some male Columbian ground squirrels have been reported to emigrate later, during the summer as two-year-olds (Festa-Blanchet and King, 1984).

Black-tailed prairie dogs are another species in which dispersal occurs later (Dobson et al., 1997; Hoogland, 1992). In this species, males do not disperse from their coteries until after they've been in their natal coterie for two years (Hoogland, 1992). This timing coincides with when their daughter would be mature enough to begin mating, thus avoid father-daughter inbreeding despite later male emigration. Additionally, female yearlings are much less likely to undergo estrous if her father is still in her natal coterie.

Causes of dispersal

Of the variety of tactics used by animals to prevent inbreeding, dispersal has been a topic of particular interest. A wide variety of hypotheses, each with varying levels of support, have been presented regarding what triggers dispersal. Included in these hypotheses are the topics aggression, population density, nest site and territory availability, food, individuals' weight and fat stores, presence of close female kin, mate availability, and offspring fitness; each will be discussed below. Importantly, no single hypothesis wholly explains dispersal in all mammal species, or, in most cases, even within a single species (Dobson and Jones, 1985; Greenwood, 1980). Animals exhibit an extensive variety of dispersal patterns, and interactions between several variables, such as inbreeding avoidance, kin competition, and kin cooperation, are necessary to explain dispersal in many species (Lawson Handley and Perrin, 2007).

Dispersal has been argued to have evolved to solve three main problems: inbreeding, intraspecific competition, and habitat instability (Bélichon et al., 1996). Evolutionarily, dispersal could have begun as a genetic error of hormone secretion in encouraging high levels of activity in a species, but because the trait is conserved across an abundance of species, taxonomic studies do not support this idea (Holekamp, 1989). More support is provided for the hypothesis that dispersal is a by-product of natural selection for males with high levels of activity; these more active males would be more likely to find mates and defend territories. Regardless of how the dispersal trait was selected, the only way it could have been maintained is if it proved to be an evolutionarily stable strategy (Lemel et al., 1997). Lemel et al. (1997) state that for dispersal to be an evolutionarily stable strategy, immigrants and residents would need to be demographically similar, and immigrants would need to compensate for any reduced fecundity through an increase in adult survival. One flaw with this argument, however, is that some research, which will be discussed below, has found that immigrants may actually have increased fecundity (Lidicker, 1962; Waser et al., 2013). Regardless of whether the details of his argument are supported by research, his hypothesis that dispersal must be an evolutionary stable strategy is certainly logical.

One dispersal hypothesis with mostly positive support is that the behavior occurs due to aggression from conspecifics (Lawson Handley and Perrin, 2007). For example, adult sciurid females are aggressive towards males during pregnancy and lactation as they defend the resources in their territory that are necessary to raise their young (Greenwood, 1980). In yellow-bellied marmots, yearlings appear to be responding to this adult female aggression because they disperse prior to the emergence of juveniles (Amitage and Downhower, 1974). During lab experiments with Richardson’s ground squirrels, adult females had more positive interactions with female yearlings, regardless of whether they were theirs or another female’s, compared to male yearlings (Michener and Michener, 1973). Field study observations resulted in similar findings in Columbian ground squirrels, except that in this species, both male and female Columbian ground squirrel adults are particularly aggressive towards male yearlings (Neuhaus, 2006; Festa-Blanchet and King, 1984). In these two species, the fact that aggression was particularly targeted at males may help explain male biased dispersal (Neuhaus, 2006; Michener and Michener, 1973). Ultimately, aggressiveness towards yearlings benefits resident adults because it reduces future competition for resources and mates (Festa-Blanchet and King, 1984).

Conversely, several studies were unsupportive of the aggression hypothesis. Juvenile males in both Belding's ground squirrels (Holekamp and Sherman, 1989) and California ground squirrels (Dobson, 1979) did not experience excessive aggression from adult males. Similarly, although round-tailed ground squirrel juvenile dispersal correlates with increased territorial behavior from adults, field observations and experiments supported that this was just a correlation, not causation (Dunford, 1977). Although adult aggression does not cause dispersal in California ground squirrels and round-tailed ground squirrels, researchers concluded that it did influence immigration. In California ground squirrels, particularly high rates of chasing from adult females towards immigrants were observed (Dobson, 1979). In round-tailed ground squirrels, so few immigrants were seen during a field study that researchers believed adults chased them out of the population so quickly that they were never even observable (Dunford, 1977). The researchers hypothesized that this aggression towards round-tailed ground squirrel immigrants may have evolved to prevent over-population in each colony.

Overpopulation prevention is another hypothesis for the cause of dispersal. In theory, emigration could occur to avoid population crashes caused by overpopulation, and those that emigrate will additionally benefit through an increased chance of survival as population density increases (Lidicker, 1962). Regardless of whether there are benefits, individuals could potentially be forced to emigrate past a certain density threshold. If this occurred, it could be that: 1) the weakest individuals will be forced to emigrate because they'll be the first to feel the pressures of declining environmental conditions and increased resource competition, or 2) the youngest, and in theory strongest, individuals will be forced the emigrate because they'll be most capable of successful emigration. Several examples in sciurid species support that population density does not affect emigration. For example, in an experimental study on Columbian ground squirrels in which several populations were monitored, population density did not affect dispersal patterns in males or females (Dobson, 1979). Research on yellow-bellied marmots, however, concluded that population density does not influence dispersal (Armitage et al., 2011).

Population density can influence food, nest site, and territory availability as individuals compete for these resources. Some researchers have suggested the hypothesis that resource availability may cause dispersal, particularly when resources in the current population are depleted or inferior to nearby populations or areas (Lawson Handley and Perrin, 2007). In supplemental food experiments, female California ground squirrels were attracted to adjacent colonies in which the food was added, thus female dispersal in the species may be related to food availability and quality (Dobson, 1979). Similarly, supplemental food experiments showed that male round-tailed ground squirrel dispersal had a tendency to increase with decreased food supply (Dunford, 1977). A meta-analysis on dispersal, however, did not support the food availability hypothesis, because, overall, food shortages did not influence dispersal in round-tailed ground squirrels and Belding's ground squirrels, and male natal dispersal was not influenced by supplemental food in California ground squirrels and round-tailed ground squirrels (Holekamp, 1984a).

Food availability does influence dispersal in female North American red squirrels, in which females are less likely to disperse in the spring, post-weaning, if they still have food stores from the previous fall (Berteaux and Boutin, 2000). When females do disperse, however, their offspring frequently take over her territory, which has been found to increase juvenile survival. Thus, in this species, dispersal seems to be a fitness trade-off between personal survival (which is influenced by food availability) and reproductive success (which is related to territory availability for offspring). Studies on Richardson's ground squirrels have yielded similar findings, in which young males retain their mother's territory if she doesn't survive hibernation and tend to disperse if she does (Michener and Michener, 1973). Research supports opposite results in black-tailed prairie dogs and yellow-bellied marmots (Holekamp, 1984a). When excessive nest burrow sites were available in populations of yellow-bellied marmots, and when black-tailed prairie dog parents vacated nest sites for their young, natal dispersal still occurred. In juvenile banner-tailed kangaroo rats, dispersers do not have superior habitats to non-dispersers (Waser et al., 2013). Overall, research supports that space availability may be a factor in dispersal for some species, but definitely not all.

A contrast to environmentally based dispersal hypotheses is the physiological one, in which dispersal occurs when a certain weight or body fat percentage is achieved; this is sometimes referred to as the ontogenetic switch hypothesis (Holekamp, 1986). Through a meta-analysis, Holekamp (1984a) determined that a combination of sex and weight may be the best predictor of dispersal. She concluded that if an individual waits until they have reached sufficient size of stored energy, they will be more likely to survive when they disperse (Holekamp, 1984a; Holekamp, 1984b). This hypothesis has ample support from studies on Belding's ground squirrels. In field studies, the body weights of juvenile male dispersers were significantly higher than males that had yet to disperse (Holekamp, 1984b). Related to that finding, in Belding's ground squirrels, when a particular body mass is reached, behavioral changes occur, including increased exploration and locomotion and decreased fear response

(Holekamp, 1986). In an experiment, when males were allowed to fatten in a lab setting to an unusually high weight, prior to hibernation, they mated during their yearling summer when typically they don't breed until two years old (Holekamp, 1984b). In addition to weight, hormones may play a role in dispersal in Belding's ground squirrels. When female pups were injected with androgens, 75% dispersed by age 60 days; this dispersal level and pattern is similar to that of males (Holekamp and Sherman, 1989).

Not all research supports the ontogenetic switch hypothesis, however. Columbian ground squirrel researchers concluded that body mass did not influence dispersal because yearling male dispersers had no difference in body mass on day 50 post-emergence as non-dispersers, and heavier individuals did not disperse earlier than lighter individuals (Wiggett and Boag, 1992). Additionally, in the five populations that were studied by Wiggett and Boag (1992), there was a wide range in body mass of dispersing individuals, and despite this, all individuals dispersed around the same time. Noteworthy, however, is that some very small individuals in this study delayed dispersal until two-years-old, rather than the typical yearling year, possibly providing support for the ontogenetic switch hypothesis that the researchers did not acknowledge in their overall conclusion.

Unrelated to body weight, a different dispersal hypothesis states that the presence of the mother or siblings will influence whether an individual disperses or emigrates. Whether that influence is hypothesized to be positive or negative depends on the study species in question, and results supporting whether presence of kin even influences dispersal is mixed. In theory, kin cooperation can encourage philopatry (Lawson Handley and Perrin, 2007), but in several sciurid species this does not seem to be the case. In Belding's ground squirrels, neither the mother's age, nor her presence, influences male hibernation location and male dispersal (Holekamp, 1984a; Holekamp and Sherman, 1989). In Richardson's ground squirrels, the presence of the mother does not influence the location of female yearlings, but does for males; males with their mother present disperse farther than males whose mothers do not survive hibernation (Holekamp, 1984a; Michener and Michener, 1973). Similar to male Belding's ground squirrels and female Richardson's ground squirrels, the presence of the mother at emergence does not increase the likelihood of emigration in Columbian ground squirrels (Neuhaus, 2006). As a contrast, in yellow-bellied marmots, the presence of their mother actually decreases the likelihood that a female will disperse (Armitage, et al., 2011).

In addition to mothers not influencing male Columbian ground squirrel emigration, the presence and sex of littermate siblings does not influence emigration (Neuhaus, 2006). Female Columbian ground squirrels with sisters present, however, tend to remain in the population, rather than "disappear" (either through death or, less likely, emigration), compared to those with no siblings or those with just brothers. Unlike Columbian ground squirrels, meadow voles of both sexes tend to respond negatively to the presence of siblings (Bollinger et al., 1993). In Bollinger et al.'s (1993) experimental study, meadow voles that were released into plots with siblings were more likely to disperse than those that were released in plots with non-siblings. Additionally, of the meadow voles that did disperse, those in plots with siblings dispersed more quickly, and while males of both groups dispersed sooner than females, the degree of sex-bias was stronger in sibling plots. A larger meta-analysis of 11 mate choice studies, including species of rodents, insects, and birds, found that in 82% of the studies (9/11), siblings avoided mating with one another (Pusey and Wolf, 1996). Another aspect of litters that may influence dispersal is size; while size and composition of litters does not influence juvenile male dispersal in

Belding's ground squirrels (Holekamp, 1984a), litter size does have a tendency to increase male dispersal in round-tailed ground squirrels (Dunford, 1977).

Dispersal when kin are present can be beneficial in that it not only minimizes inbreeding, but it also could lead to an increase in mates as males move from one area to the next (Lidicker, 1962; Holekamp and Sherman, 1989). In addition to increasing the number of mates for the individual, the trait for dispersal, if variable and genetic, would become more prevalent due to the increased mating success and offspring production of dispersers, thus spreading the trait (Lidicker, 1962). Research has also supported the hypothesis that competition for mates influences dispersal, specifically in polygynous and promiscuous species (Dobson, 1982). An alternative hypothesis, however, is that immigrant males have, overall, fewer mates than residents because they lose mating opportunities during the dispersal period (Olson et al., 2012; Waser et al., 2013). Despite the variety of hypotheses about how mating opportunities could influence dispersal, several studies in ground squirrel species have supported that the two variables may not be related. For example, in Belding's ground squirrels, males that disperse have equivalent access to mates as non-dispersers (Holekamp and Sherman, 1989). In banner-tailed kangaroo rats, dispersers have no advantage in gaining mates when compared to philopatric individuals (Waser at al., 2013). And in California ground squirrels, researchers concluded that competition for mates did not influence dispersal because dispersal of young males occurred despite a lack of adult males, who would have been competitors (Dobson, 1979).

The conclusions of the studies above, however, only compare the quantity, not the quality or compatibility, of mates. Who the mate is can be just as important as the number of mating partners, especially in light of inbreeding depression and female cryptic mate choice, described previously. Hypothetically, individuals that emigrate will have offspring with higher fitness because those offspring will have higher heterozygosity and a higher probability of creating new, advantageous genetic combinations as a result of outbred parents (Lidicker, 1962). Waser et al. (2013) explored this hypothesis in their study on banner-tailed kangaroo rats. They found that dispersal had no fitness benefits, including no differences in offspring production or offspring survival when compared to non-dispersers, and no reduction in inbreeding depression.

Goals, hypotheses, and predictions

The information above discusses ideas related to two main questions: 1) do animals avoid inbreeding, and 2) if they do avoid inbreeding, what mechanisms do they use to do so? Columbian ground squirrels are an appropriate model for addressing inbreeding avoidance for both practical and biological reasons. Practically speaking, they're abundant, diurnal, easy to trap and mark, and often live in areas that make them easily visible, like plains and meadows (Holekamp, 1984a). Behavioral interactions, like matings, basic biological measurements, such as weights and dates of emergence from hibernation, and pedigree information can be reliably and carefully monitored from year to year, resulting in a rich dataset.

Biologically, Columbian ground squirrel females are philopatric, remaining within their natal population throughout their lifetime (Murie and Harris, 1984). Males, however, exhibit mixed dispersal patterns; some males emigrate to a new population as yearlings (or two-year-olds if lightweight), while other males remain philopatric residents in their natal colony, like the females (Murie and Harris, 1984; Festa-Blanchet and King, 1984). The reported levels of male philopatry for this species are varied; one eight-year study recorded 17% of males remaining philopatric residents (Murie and Harris, 1984) while a different study over eight years found

70% of the adult males to be philopatric residents in the population, and only 65% of the residents emigrating as yearlings (Neuhaus, 2006). Due to this variable dispersal pattern, in which both females and some males remain residents, populations of this species have a high potential for inbreeding. Columbian ground squirrels are an apt study species to examine inbreeding because, in addition to exhibiting moderate levels of male philopatry, they are polygynandrous, with both females and males mating with multiple partners, and multiple paternity occurs, in which a single litter may have multiple fathers (Murie, 1995; Raveh et al., 2011). Multiple matings and multiple paternity may help with investigating the inbreeding avoidance techniques described in previous sections.

The goal of this study was to determine the effects of the presence of close female kin on the mating habits of male Columbian ground squirrels. We additionally aimed to determine whether inbreeding occurs in this species and, if not, what mechanisms the ground squirrels have to avoid inbreeding. To achieve these goals, we proposed a series of hypotheses to explain the male dispersal pattern present in Columbian ground squirrels, in which some males remain philopatric and others emigrate, and to determine and explain any inbreeding that occurs in this species. These hypotheses are based on the inbreeding avoidance mechanisms described above, particularly individual recognition, mating opportunities, and dispersal.

Hypothesis 1: The males that are philopatric residents remain in the population because they have no surviving, closely related, female relatives (specifically mothers and littermate sisters), and thus, they are not triggered to emigrate to avoid inbreeding.

Prediction 1: Adult, resident males do not co-occur with their mother or littermate sisters.

Hypothesis 2: Although philopatric resident males have close female relatives present (mother and littermate sisters), they recognize that female relatives are present and don’t mate with any females. They are not triggered to emigrate to avoid inbreeding.

Prediction 2: Resident males do not mate when they co-occur with their mother or littermate sisters.

Hypothesis 3: Although philopatric resident males mate when they co-occur with close female relatives (mother and littermate sisters), they are able to recognize those relatives and do not mate with them. They are not triggered to emigrate to avoid inbreeding.

Prediction 3: Resident males do not mate with their mother or littermate sisters.

Hypothesis 4: Although philopatric resident males mate with their close female relatives (mothers and littermate sisters), they produce no offspring with them. Due to this, they are not triggered to emigrate to avoid inbreeding.

Prediction 4: Resident males mate with their mothers and littermate sisters but produce no offspring with them.

Hypothesis 5: Inbreeding between philopatric resident males and close female relatives (mothers and littermate sisters) results in viable offspring. Resident males are not triggered to emigrate because they do not need to avoid inbreeding due to their ability to produce viable offspring during inbreeding.

Prediction 5: Resident males mate with their mothers and littermate sisters and produce offspring with them at success levels that are similar to those of immigrant males.

Methods

Study species and site

Columbian ground squirrels (*U. columbianus*) are colonial, diurnal, ground-dwelling sciurid rodents that live in alpine and sub-alpine meadows of the Rocky Mountains in the north-western United States and south-western Canada (Elliot and Flinders, 1991). They are polygynandrous in matings and exhibit multiple paternity (Raveh et al., 2011; Murie, 1995). We studied a population of Columbian ground squirrels in Sheep River Provincial Park, Alberta, Canada (110°W, 50°N; 1550 m elevation) during their active season (approximately mid-April to early August) from 1992 to 2015. The population was located in a meadow with several surrounding meadows with other Columbian ground squirrel populations. We used data collected from the years 2005 to 2015 (the years in which mating log information was collected and paternity was estimated for offspring, see below). We collected data for 56 individual males as adults aged two years and older (some of which lived in the population as adults multiple years, therefore analyzed as a sample of 127 adult male-years) and 90 individual adult mating females (some of which mated in the population as adults multiple years, therefore analyzed as a sample of 244 adult mating female-years, 191 of which had mating logs). Random effects models measuring the variance of number of mates and number of offspring for each male showed low repeatability among years (see results below), therefore each yearly sample was treated as an individual male and individual female in the analyses of this study, rather than averaging values across years for each ground squirrel. The male samples and female samples are referred to as "male samples" and "female samples" for simplicity.

Columbian ground squirrels hibernate for approximately eight to nine months of the year (Michener, 1984; Dobson and Murie, 1987; Dobson et al., 1992). Hibernation begins in mid-July to late August with exact date for an individual dependent on several variables, including age, sex, the date of emergence from hibernation that year, and whether a female produced offspring (Michener, 1977). Late summer, prior to hibernation, is when some young males, typically yearling aged, emigrate to other populations, if they emigrate at all; juveniles, however spend their first hibernation in the natal population (Neuhaus, 2006). Reports of the proportion of Columbian ground squirrels that emigrate vary. Neuhaus (2006) found that 65% of yearling males and 35% of yearling females of the species "disappear," while Murie and Harris (1984) estimated that 83% of males and 13% of females emigrate. When we first observed each immigrant male in the population, at the beginning of the active season, we recorded him as a two-year-old, although particularly heavy males (over 500 g) were occasionally listed as three-year-olds.

At the start of the active season, adult males emerged first (first emergence date ranging from April 11th to April 26th, n=11 years), followed by adult females aged 1 year and older (first emergence date 3.5±2.7 SD days later on average, n=11 years), and yearling males (first emergence at 11.0±5.1 SD days later than adult males on average, n=11 years). Upon emergence, we identified individuals by their pair of numbered, metal ear tags (Monel #1; National Brand & Tag Co., Newport, KY, USA) that were attached to their ears at first emergence as juveniles. We trapped each individual using live, single door traps baited with peanut butter (sized 15×15×48 and 13×13×40 cm3; Tomahawk Live Trap Co., Tomahawk, WI, USA) and marked each individual with a unique symbol on the dorsal pelage, for visual identification, using black hair dye (black pearl shade of Hydrience Clairol brand; Proctor and

Gamble, Stamford, CT, USA). We also recorded emergence date, emergence location, and weight to the nearest 5 g using Pesola spring-slide scales. We determined emergence locations using an x-y coordinate (Cartesian) grid that was placed over the meadow. The grid was made of colored flags approximately 0.5 m tall and set at 10 m intervals. Individuals were periodically re-trapped and re-marked, when dye marks faded, and re-weighed throughout the active season, particularly females during gestation and at the end of lactation (near the time of weaning of offspring).

Mating sequence

Soon after emergence (4.1±2.2 SD days, n=222 females samples), females underwent a single estrous lasting a few hours (usually 4-6 hours) on a single day (Elliott and Flinders, 1991). During this time, adult females (aged 2 years and older and rarely 1-year-olds) mated with multiple males (3.7±1.4 SD males, range 1-8, n=191 female samples), and adult males (aged 2 years and older) mate with multiple females (6.2±5.2 SD females, range 0-18, n=115 male samples). To determine when a female was ready to mate, females were caught daily to look for signs of imminent estrous, including the presence of vulvar swelling, openness, and vaginal fluid (Murie, 1995; Murie and Harris, 1982).

Mating behavior was observed between 06:00 and 19:00 hours. Squirrels were identified by their dye mark. All female yearlings and adults and all males 2 years and older were observed for potential mating behaviors (see below). For each female’s mating log, we recorded: 1) sequence of mates, 2) mating locations, 3) mating duration, and 4) when present, mate guarding duration.

Prior to estrous, we made detailed notes of locations and interactions between adult males and females and estimated male territories; this allowed us to determine potential mating partners and increase the accuracy of mating sequence records. We took these notes using 10 minute interval scan sampling of the locations and behaviors of all visible individuals and using focal sampling of individuals of interest.

To estimate actual mating partners, we noted which males frequently slept in the same burrows of recently emerged females; these males were often a female's first mating partner (Raveh et al., 2011; Nesterova et al., 2011). We inferred subsequent mating partners through observations with binoculars either on the ground or on wooden stands (approximately 3 m high) throughout the meadow. During mating, all individuals of both sexes are capable of accessing the entire meadow, and therefore able to access every other individual in the meadow, regardless of where they are located (F. S. Dobson and C. Klase, personal observations). Signals that a male and female may soon mate included: frequent sniffing of the genital area by a potential male partner, chasing of the female by the male, and chasing of the male by the female; these signals could occur prior to or during the day of estrous (Harris and Murie, 1984; Murie and Harris, 1982; Murie, 1995). During estrous, a male mating partner often trapped the female in a burrow, followed by mate guarding (Murie and Harris, 1982; Manno and Dobson, 2008). Because mating occurs underground, a male was considered a female's partner if the two entered the same burrow and stayed below ground together for at least seven minutes during estrous. A female's mating behaviors were observed until she no longer accepted advances from mates during her estrous. Underground consortships and mate guarding occurred only on the day of a female's estrous (Manno and Dobson, 2008).

Observations of male post-mating guarding behavior of female mates were used as an additional sign that mating had occurred. Mate guarding occurred when a male maintained close proximity to a female and prevented other males from interacting with the female, either by chasing the female and/or by pushing or chasing the female mate into a burrow (thus, effectively preventing her from leaving the local area and mating with additional males). While guarding, the males frequently made constant and repeated alarm vocalizations and also chased and fought with other approaching males (Manno et al., 2007). Another indication that mating had occurred was grooming of the genitals by the male and/or female and, for females, mating of hair with copulatory plug material, presence of a vaginal plug, or removal of a vaginal plug (Murie and Harris, 1982; Murie, 1995; Raveh et al., 2011). Some mating sequences were missed due to weather that prevented observations or individuals mating outside of visibility range. Mating logs for such females were not included in the study. We recorded mating logs for 191 female samples out of 244 female samples that mated during the 11-year study period. We assumed this to be an unbiased sample of mating sequences.

To confirm mating partners and sequences, post observations, DNA paternity tests were performed for all offspring. These tests were particularly beneficial in confirming that the male sleeping partner of a female, during the night prior to estrous, was a mating partner; the first mate (often the sleeping partner if a female had one) sires the most offspring in a litter (Nesterova et al., 2011; Raveh, et al., 2010). Through these paternity tests, mistakes (males siring offspring with females with which they were not observed mating) were found in 46 of the 191 mating logs of females samples, with 58 instances of male samples found to be fathers when they were not initially observed mating. These male mating partners determined through DNA paternity tests, rather than field observations, were added to the mating logs for data analysis.

Due to the error rate and necessary additions of mating partners post field observations, mating sequence was not analyzed for this study.

Gestation, birth, maternity, tagging, and paternity

After mating, pregnant females gestate an average of 24.7±0.8 SD days (n=198 females samples). During this time, we periodically trapped and checked females for pregnancy status (signs included weight gain and physically feeling in utero fetuses through abdomen palpations). Behavioral signs of pregnancy included nesting (creation of a burrow with a discrete opening in which nest materials, such as dried grass and twigs, are placed) and aggression towards other ground squirrels near the nest burrow (Murie, 1995). Nest burrows were marked with a colored flag for future reference.

Approximately 22 days post mating, we caught females exhibiting signs of pregnancy in live traps in the field and brought them into a field laboratory to give birth. Through this method, and field observations at juvenile emergence, we were able to determine maternity for each individual. While in the laboratory, females stayed in single occupancy, clear, polycarbonate microvent rat cages with a wire, angled top (48×27×20 cm^3; Allentown Caging Equipment Company, Allentown, NJ, USA). To mimic burrow conditions, boxes were covered in black plastic bags (to maintain darkness) and contained shredded newspaper and pine chip bedding for nesting materials. Twice daily, females were feed an ad libitum mixture of apples, lettuce, and horse feed (EQuisine brand; Okotoks, Alberta, Canada).

Several times a day we visually checked boxes for pups using a flashlight. Of the females that mated, 89.2% produced litters, and those litters had an average of 3.3±0.9 SD pups (n=198 litters). Pups are born hairless with closed eyes and ears, with an average mass of 11.83±1.52 SD g (n=654 juvenile samples), and are completely dependent on their mothers for nutrition and protection (Shaw, 1925). Within 12 hours after birth, we weighed pups to the nearest 0.01 g on an electronic scale and determined their sex via visual observation of external genitalia. Additionally, we used sterile scissors to biopsy skin tissue from an outer hind toe (either left, right, or both depending on the number of pups and their sex); the missing tissue left a scar that aided later identification of pups in the field. Biopsied tissues, as well as a short tail biopsy of approximately 1 mm, were preserved in 95% EtOH for use in microsatellite DNA analysis of paternity. For DNA analysis, 13 microsatellite loci were amplified using polymerase chain reaction (PCR) for each individual, and CERVUS 3.0 (Marshall et al., 1998; Kalinowski et al., 2007) was used to assign paternity (further analysis details described in Raveh et al., 2010). If birth occurred in the morning, we released the mother and pups into the field that afternoon, and if the birth occurred in the evening, we released them the next morning (release details described in Murie, et al., 1998).

After release, pups remain underground in nest burrows with their mother, continuing physical development, for the next 26.5±1.1 SD days (n=152 litters) on average. After that time, they emerge as juveniles, with an average mass of 107.12±21.03 SD g (n=505 juvenile samples), and they begin independently eating and taking trips that are gradually further from the nest burrow (Shaw, 1925). Of the litters produced, 87.4% of them had one or more pups that survived to juvenile emergence. Litters that successfully emerged had an average of 2.9±1.1 SD pups (n=173 litters) at emergence, and all litters combined had an overall sex ratio at emergence of 1.1 males to 1 female (n=499 juvenile samples). Pups from a litter emerged on the same day, typically within a few hours of each other. Once all pups from the litter had emerged, we recorded the location of emergence (using the x-y coordinate system) and weight to the nearest gram using a spring scale. We marked each individual with a unique dye mark on the dorsal pelage, attached numbered, metal tags to the ears, and, using sterilized scissors, collected a small biopsy sample from the distal portion of one pinna.

Data Analyses

We performed all calculations, unless otherwise specified, in R statistical program version 3.0.1 (R Core Team. 2013). For data analysis, close female relatives included only littermate sisters and mothers. Littermate siblings are only 39% genetically related due to multiple paternity; litters contain a mixture of paternal full and half siblings. (Raveh et al., 2010). Previous research, however, suggests that Richardson's ground squirrels (Davis, 1984) and Columbian ground squirrels (King, 1989; Viblanc et al., 2010; Dobson et al., 2012) interact differently with uterine and non-uterine kin, and Columbian ground squirrels are able to distinguish close kin by scent (Raynaud and Dobson, 2011; Steiner, 1974).

To check that we could analyze each male and female sample as an individual, we performed random effects models with male ID as the random effect. Number of mating partners was the measured variable in one model, and number of offspring produced was the measured variable in the other. Through these tests, we measured the variance of the number of mating partners and number of offspring for each individual male sample to check for repeatability levels. Based on the results, each sample was treated as an individual in this analysis.

To determine if the presence of close female relatives influenced male emigration, we performed two Pearson's chi-squared tests, with Yates' continuity correction, with the 71 resident adult (two years and older) male samples and 240 adult female samples that mated. One chi-squared compared co-occurrence with mothers and sex of their offspring, and the other compared co-occurrence with littermate sisters and the sex of their co-occurring littermate. Female samples acted as a comparison group for the male samples under the premise that, because females remain residents throughout their lives, if males emigrate due to their close female relatives, they should co-occur at lower rates than females. To further investigate the results of the chi-squared test of sex and littermate sister co-occurrence,

We additionally compared characteristics of male samples that co-occurred with one or more littermate sisters and those that didn't using Welch two sample t-tests with the 71 adult resident male samples. Traits analyzed were age in years, emergence date (measured as number of days the individual emerged after the date of the first individual's emergence that year), and mass (in grams) at emergence.

We determined if the presence of close female relatives influenced the mating patterns of male samples by performing two Fisher's exact tests; this test was selected due to low sample sizes in some groups. Both Fisher's exact tests used the 70 resident adult male samples with confirmed mating status. One test compared co-occurrence with mother and whether the male sample mated that year, and the other compared co-occurrence with one or more littermate sisters and whether the male samples mated that year. For the Fisher's exact test on co-occurrence with littermate sisters, individual male and females samples were included regardless of whether they had a littermate sister present at birth; males and females that had no littermate sisters at birth were included in this analysis. We assumed, however, that because the ratio of males to females at emergence was even (1.1 males: 1 female), variations in presence of littermate sisters would be similar between the two sexes and not affect the test.

To further examine the results of the Fisher's exact tests of co-occurrence with mother and presence of mating, we performed three ANOVAs (for age, mass, and emergence date) using the same sample of 70 resident adult male samples. We also performed two Welch two sample t-tests: one compared the number of offspring of resident adult male samples that mated and co-occurred with their mother (n=17 male samples) and those that mated and didn't co-occur (n=41 male samples), and the other compared the number of mates of resident adult male samples that mated and co-occurred with their mothers (n=14 male samples) and those that mated and did not co-occur (n=37 male samples). Similarly, we performed an ANOVA using mass as the dependent variable to accompany the results of the Fisher's exact test on co-occurrence with littermate sister and presence of mating and also performed two Welch two sample t-tests. In these t-tests we compared the number of offspring produced (n=58 male samples) and compared the number of mates (n=51 male samples) of male samples that mated and co-occurred with one or more littermate sisters (n=6 and 5 males samples, respectively) and male samples that mated but did not co-occur with littermate sisters (n=52 and 46 males samples respectively). For t-tests on offspring produced and on number of mates, we only included male samples that mated; for tests on number of mates, male samples from 2013 were additionally excluded because we did not collect mating logs that year.

We performed several analyses to determine the effects of mass and age on mating likelihood in this population to further investigate the results of the Fisher's exact tests and ANOVAs. We created a binomial, generalized linear model of the effect of mass on mating likelihood and a polynomial regression model of the effect of age on weight. For the first model we used the sample of 70 adult resident male samples with known mating statuses and for the second we used all 71 resident adult male samples. Using two Welch two-sample t-tests, we compared the number of mates (n=22 male samples) and also the number of offspring produced (n=27 male samples) between two-year-old resident male samples (including only those who mated; n=8 and 11 male samples, respectively based on test) and three-year-old male samples (n=14 and 16 males samples, respectively based on test). This was followed up by two models on resident male samples aged three years and older: one addressed the relationship between age and number of mates (n=43 male samples) through a polynomial regression, and the other measured the relationship between age and offspring (n=47 male samples) through a linear regression. Males from 2013 were excluded in the analyses that involved number of mates due missing data.

We performed a Pearson's chi-squared test with Yates' continuity correction comparing observed and expected values of potential and actual mating pairs to determine if the relationship to a potential mate influenced whether mating actually occurred (i.e. if Columbian ground squirrels avoided incest). Related pairs were defined as male-closely related female pairs (i.e. son-mother and brother-littermate sister), and unrelated pairs were pairs of any other familial relationship and of no known relationship. For this test, we included all resident adult male samples and all adult female samples with known mating logs. All possible pairing combinations each year were calculated (n=2228 pairs total), and all pairs that were observed mating were summed.

To determine more detail about the results of this chi-squared test on the effect of pair relatedness on pair mating, we created random mating models (using Microsoft Excel, 2010) and calculated mating probability of each possible pair in the population during the study period. For each individual year, we determined all possible mating pairs and assigned each pair a number. We summed the number of observed mating pairs that year and randomly selected pairs to replace the summed observed pairs. This procedure was designed to best mimic the polygynandrous mating system of the species, in which each individual can mate with multiple individuals per year. We matched the number of random pairs to the number of observed mating pairs to best simulate the mating situation each year. When the number of mates of female samples was unknown (i.e. mating sequence for female was not recorded), the average number of mates of that year was multiplied by the number of female samples with unknown mates, and we added that number to the total observed mating pairs of that year. We repeated the procedure of random replacement 10,000 times for each year. Pairs were allowed to "mate" multiple times in each iteration, although multiple matings between a male sample and female sample within the same year was not taking into account for actual observed mating counts. Multiple matings were allowed in the randomization simulation because each pair was given equal opportunity for this to occur, and we therefore assumed multiple matings had little effect on mating probabilities. We summed the number of iterations in which each pair mated during and divided that by the total number of iterations minus 1 (9,999) per year to determine the probability of each pair mating that year. If dis-assortative mating had occurred, and close relatives (defined as mother-son pairs and brother-littermate sister) avoided mating with each other, the actual instances of mating will, overall, differ from the probabilities.

Results

Repeatability: mates and offspring

Random effects models measuring the variance of the number of mating partners within each individual male (n=54 individual males; n=113 male samples) between the years present indicated low repeatability. We observed that for number of mating partners, the repeatability was 0.079 (among group variance =2.166 [±2.42; ±95% CI], total variance = 27.411). Similarly, for number of offspring produced (n=56 individual males, n=127 males samples), the repeatability was also low at 0.093 (among group variance= 3.762 [±6.11; ±95% CI], total variance = 40.283). For both variables, because the confidence interval for the among-group variance overlapped zero, the repeatability was judged to be extremely low.

Co-occurrence

Of the 127 adult male samples, 71 (55.9%) were philopatric residents. Of the 71 male resident samples, 33 (46.5%) co-occurred with a close female relative (mother and/or littermate sister(s). Out of the 240 adult female samples, 140 (58.3%) co-occurred with a close female relative.

A Pearson's chi-squared test (with Yates' continuity correction) of adult male samples and adult female samples co-occurrence with mothers indicated no significant difference between observed and expected values (Pearson's χ^2 =2.633, d.f.=1, n=311, p=0.105; Table 1.1). Sex and co-occurrence with mother were not significantly dependent variables, and according to the chi-squared test there was a minimal difference in the likelihood of male samples (36.6%) and female samples (25.8%) co-occurring with their mother.

Sex and expected co-occurrence with one or more littermate sisters, compared to observed co-occurrence, however, had a statistically significant difference. Female samples were 1.86 times more likely to occur with littermate sisters (28.8%) compared to male samples (15.5%) (Pearson's χ^2=4.370, d.f.=1, n=311, p=0.037; Table 1.2). Although sex and co-occurrence with littermate sisters were significantly dependent, the association was weak (φ=0.119).

We found some noteworthy differences between adult resident male samples that co-occurred with adult littermate sisters and those that didn't. A Welch two sample t-test found no significant differences between emergence dates of adult resident male samples that did not co-occur with one or more adult littermate sisters and those that did (5.6±7.2 SD days compared to10.4±10.4 SD days, respectively; d.f.=11.81, 95% CI [-11.91, 2.42], p=0.174; Figure 1.1), but adult resident male samples without co-occurring adult littermate sisters were found to be an average of 1.5 years older (3.9±1.8 SD years compared to 2.4±0.7 SD years, respectively; d.f.=42.64, 95% CI [0.92, 2.36], p<0.001; Figure 1.2) and weigh an average of 81.7 g more (517.6±76.8 SD g compared to 435.9±75.8 SD g respectively; d.f.=14.04, 95% CI [28.26, 135.09], p=0.005; Figure 1.3) that those with co-occurring adult sisters.

Co-occurrence with mother and male mating

Of the 70 adult resident male samples with known mating status, 25 (35.7%) co-occurred with the mother and 10 (14.2%) co-occurred with one or more littermate sisters. A Fisher's exact test of resident adult male samples co-occurring with their mothers and resident adult male samples that did not co-occur indicated a significant difference between observed and expected values of male sample mating (d.f.=1, n=70, 95% CI [1.09, 24.32], p=0.021, Table 1.3). Residuals indicate that more male samples co-occurring with their mothers were expected to mate that year if co-occurrence with mother and mating were independent variables. Male samples without co-occurring mothers were 1.34 times more likely to mate with one or more females (91.1%) compared to those with co-occurring mothers (68.0%).

When ANOVA with post-hoc Tukey's HSD test comparisons were calculated, significant differences were found between the four co-occurrence with mother-mating groups for the variables of age (d.f.=3, F=12.57, p<0.001; Table 1.4, Figure 1.4), mass (d.f.=3, F=10.57, p<0.001; Table 1.5, Figure 1.5), and emergence dates (d.f.=3, F=18.37, p<0.001; Table 1.5, Figure 1.6). A Tukey's HSD post hoc test indicated that the average age of male samples that did not co-occur with their mothers and did mate (4.6 SD±1.8 years) was statistically significantly greater than the average age of male samples in all other groups: male samples that co-occurred and mated (2.8±0.8 SD years; p<0.001), male samples that co-occurred and did not mate (2.0±0.0 SD years; p<0.001), and male samples that did not co-occur and did not mate (2.0±0.0 SD years; p=0.007). Male samples that co-occurred with their mothers and did mate had no statistically significant difference in age than male samples that co-occurred and did not mate (p=0.622) and male samples that did not co-occur and did not mate (p=0.786).

The average mass of male samples that did not co-occur with their mothers and did mate (539.2±67.6 SD g; Table 1.5, Figure 1.5) was statistically significantly greater than male samples that co-occurred and did not mate (428.8±77.1 SD g; p<0.001) and male samples that did not co-occur and did not mate (392.5±32.8 SD g; p<0.001) but was not significantly different that male samples that co-occurred and did mate (490.9±71.1 SD g; p=0.078). The average mass of male samples that co-occurred and did mate was also not statistically significantly greater than male samples that co-occurred and did not mate (p=0.157) but was nearly significantly greater than those that did not co-occur and did not mate (p=0.056).

The average number of days for emergence after the first individual of the season was significantly different for male samples that did not co-occur with their mothers and did mate (4.0±6.0 SD days; Table 1.5, Figure 1.6) compared to male samples that co-occurred and did not mate (15.3±7.8 SD days; p<0.001) and those that did not co-occur and did not mate (22.5±9.0 SD days; p<0.001) but was not significantly different than male samples that co-occurred and did mate (4.1±3.9 SD days; p=0.999). Male samples that co-occurred and mated emerged statistically significantly earlier than male samples that co-occurred and did not mate (p<0.001) and male samples that did not co-occur and did not mate (p<0.001).

A Welch two-sample t-test found that male samples co-occurring with their mothers that did mate had no statistically significant difference in the number of mates (7.4±4.1 SD mates) when compared to male samples that did not co-occur with their mothers and mated (9.7±3.8 SD mates) (d.f.=22.03, 95% CI [-4.94, 0.33], p=0.084; Figure 1.7). Similarly, there was also no statistically significant difference in number of offspring produced of male samples that co-occurred with their mothers and mated (6.4±6.5 SD offspring) and those that did not co-occur with their mothers and mated (7.9±5.6 SD offspring) (d.f.=26.19, 95% CI [-5.24, 2.19], p=0.406; Figure 1.8).

Co-occurrence with littermate sister and male mating

Similar to how the presence of the mother significantly affected whether an adult resident male sample mated, co-occurrence with one or more littermate sisters also appeared to significantly affect whether an adult resident male sample mated. A Fisher's exact test of adult male samples co-occurring with one or more littermate sisters and those not co-occurring with littermate sisters indicated no significant difference between observed and expected values of mating, however the difference approached significance (d.f.=1, n=70, 95% CI [0.71, 23.00], p=0.061; Table 1.6), and male samples without littermate sisters present were 1.45 times more likely to mate with one or more female samples (86.7%) compared to those with littermate sisters present (60%).

When an ANOVA was calculated for mass, significant differences were found between the four littermate sister co-occurrence-mating groups (d.f.=3, F=9.71, p<0.001; Table 1.7, Figure 1.9). A Tukey's HSD post hoc test indicated that the average mass of male samples that did not co-occur with one or more littermate sisters and did mate (529.6±71.6 SD g) was statistically significantly greater than male samples that co-occurred and did not mate (371.3±43.5 SD g; p<0.001) and male samples that did not co-occur and did not mate (439.4±65.8 SD g; p=0.006) but had no statistically significant difference in average mass compared to male samples that co-occurred and mated (485.0±62.1 SD g; p=0.447). Male samples that co-occurred with one or more littermate sisters and mated had a near statistically significant difference in average mass compared to male samples that co-occurred and did not mate (p=0.062) but no statistically significant difference compared to male samples that did not co-occur and did not mate (p=0.617).

Of the 11 samples of males that co-occurred with their littermate sisters, eight samples (72.7%) were of two-year-olds, and six of those eight (75.0%) were no longer in the population the following year (either due to death or emigration). Of those six samples that were only in the population as two-year-olds, the mating status of five was known, and only two of those five mated (one sample mating six times and producing three offspring and the other mating only once and producing only one offspring). Only two individuals that co-occurred with one or more littermate sisters remained in the population past age two years; these two contributed to five of the 11 instances of co-occurrence with one or more littermate sisters and were four of the six instances of males co-occurring with their littermate sister and mating.

A Welch two sample t-test found no statistically significant difference in the number of mates between male samples that co-occurred with one or more littermate sisters and mated (6.0±4.1 SD mates) and those that didn't co-occur and mated (9.4±3.9 SD mates) (d.f.=4.81, 95% CI [-8.46, 1.59], p=0.138; Figure 1.10). There was also no statistically significant difference in the number of offspring produced between male samples that co-occurred with one or more littermate sisters and mated (6.3±5.9 SD offspring) and those that did not co-occur and mated (7.6±5.9 SD offspring) (d.f.=6.23, 95% CI [-7.35, 4.90], p=0.644; Figure 1.11).

Inbreeding

Between the years 2005-2012 and 2014-2015, when all resident male samples two years and older and all female samples with mating logs were included, there were 2228 potential mating pairs. Of these pairs, 2200 (98.7%) were of unrelated individuals, in which the male sample and female sample were neither mother-son nor brother-littermate sister, and 28 (1.3%) were either mother-son or brother-littermate sister pairs.

A Pearson's chi-squared test with Yates' continuity correction found a statistically significant difference between observed and expected values of confirmed matings of these pairs (Pearson's χ^2 =4.526, d.f.=1, n=2228, p=0.003; Table 1.8). Non-related pairs mated 8.83 times more often (700/2200 pairs; 31.8% of the time) than related pairs (1/28 pairs; 3.6% of the time). Although mating and pair relatedness were dependent variables, the association was weak (φ=0.063).

Random mating models found the average probability of male samples mating with their close females relatives (mothers or littermate sisters) to be 25.8%±4.5% SD (range 19.7%-33.7%, n=28 pairs; Table 1.9). This modeling indicated we would expect 7.2 pairs of the 28 to mate if no dis-assortative mating occurred, while the Pearson's chi-squared predicted 8.8 pairs to mate if relatedness and mating were independent variables. Of the 28 related pairs, only one instance of close inbred mating (3.6% of the related pairs) was confirmed, and although the female samples produced two offspring, her son was the father of neither. This pair had a 33.2% chance of mating, according to randomization models and was comprised of a 3-year-old male and his mother in 2010; during that year, only three adult males were present, and only two of the three mated. That particular male mated with 11 females of the 17 females with mating logs; mating logs for four females were not recorded. Females mated with an average of 1.4±0.5 male samples (n=17 female samples) that year. The average number of adult males present each year, including both residents and immigrants, was 11.5±4.6 males (range 3-17 males; n=11 years), and the average number of mating males per year was 8.2±3.8 males (range 2-13 males; n=10 years). Thus, in addition to having a higher than average probability of mating, the year during which this related pair mated had the lowest number of males present and the lowest number of male samples that mated.

Effects of weight and age on mating likelihood

Through a generalized linear model, we found that for each 1.0 g increase in mass, adult resident male Columbian ground squirrels samples were 1.02 times (1.01, 1.04; 95% CL) more likely to mate with one or more females (n=70, p<0.001). Of the 70 resident adult male samples with known mating status, 15.7% were two-year-olds. Although 100% of all male samples aged three years and up mated, only 47.8% (11 of the 23 two-year-old males) mated.

A polynomic regression model of the effect of age on weight showed that for adult resident male samples, for every one-year increase in age, there was a 114.5 g (±33.50; ±95% CI) increase in mass (r^2=0.57, d.f.=68, p<0.001; Figure 1.12) with mass peaking at age six and a polynomic curve of a 9.1 g (±3.51; ±95% CI) decrease in mass (p<0.001).

A Welch two sample t-test showed three-year-old resident male samples had a statistically significantly greater average number of mates (9.9±2.7 SD mates) compared to mating two-year-old male samples (2.8±2.1 SD mates) (d.f.=17.77, 95% CI [-9.37, -4.99], p<0.001; Figure 1.13). Similarly, three-year-old samples had a significantly greater number of offspring (9.6±6.6 SD offspring) compared to mating two-year-old samples (1.9±1.7 SD offspring) (d.f.=17.82, 95% CI [-11.33, -4.10], p<0.001; Figure 1.14). A polynomic regression model of the effect of age on number of mating partners showed that for resident male samples aged three and up, for every one-year increase in age, there was a 3.0 mates (±3.64; ±95%) increase in number of mating partners, however neither the effect of age (p=0.104), nor the polynomic curve (p=0.079), were statistically significant (r^2=0.04, d.f.=40, p=0.160; Figure 1.15). A general linear model of the effect of age on offspring indicated that for resident male samples aged three and up, for every year increase in age, there was a 0.7 offspring (±1.02; ±95% CI) decrease in offspring produced, however this results was not statistically significant (r^2=0.03, d.f.=45, p=0.145; Figure 1.16).

Discussion

Co-occurrence

Due to the myriad of negative consequences of inbreeding described in the introduction, our hypotheses were developed under the expectation that individuals in the Columbian ground squirrel population would avoid inbreeding. Single sex dispersal and dispersal prior to reproductive maturity in particular can minimize the possibility of inbreeding (Pusey; 1987; Greenwood, 1980). Columbian ground squirrel males, similar to many mammalian species, exhibit male biased dispersal as well as dispersal typically occurring as yearlings (sometimes as two-year-olds if light mass), prior to sexual maturity (Festa-Blanchet and King, 1984; Wiggett and Boag, 1989; Neuhaus, 2006). Based on these dispersal details, we first hypothesized that inbreeding would be avoided in Columbian ground squirrels through males with close female relatives dispersing to avoid inbreeding, and only males without close female relatives remaining in the population. Our findings did not support this in regards to co-occurrence with mothers, but did with sisters. Males co-occurred with mothers at similar rates as females, but females were 1.86 times as likely to co-occur with littermate sisters. We next hypothesized that males avoid close female relatives by not mating with any individuals when co-occurring with close female relatives. Although male samples without co-occurring mothers were 1.34 times more likely to mate with one or more females compared to male samples without co-occurring mothers, and male samples without co-occurring littermate sisters were 1.45 times more likely to mate, there were still some males mating when co-occurring with close female relatives.

Therefore, the hypothesis was not fully supported. We then addressed the next hypothesis that males did co-occur with close female relatives but inbreeding would be avoided, possibly by recognition of close female relatives. Our results supported this, with only one instance of inbreeding occurring out of 28 possible inbreeding opportunities, despite the prediction from the randomization mating model and Pearson's chi-squared that either 7.2 or 8.8 pairs (depending on the test) of those 28 pairs should mate. Additionally, unrelated pairs of males and females mated 8.83 times as often as related pairs. If we had found that males did mate with close female relatives, we hypothesized that males and close female relatives would be unable to produce offspring. In the one instance of inbreeding, the mother did not produce offspring from her son, despite the fact that she did produce offspring from an immigrant male. Finally, we proposed a null hypothesis that males and close female relatives both mate and produce offspring, and dispersal is not necessary because the offspring incur no negative effects from inbreeding. Our sample of a single instance of close kin mating was not sufficient for testing this prediction.

Murrie and Harris (1984) found in their study on Columbian ground squirrels that only 17% of males remain philopatric. Had that been the case for our population, the chances of close inbreeding would be relatively minimal, assuming that philopatric and immigrant males were equally likely to breed. We found that 56% of male samples in the population were philopatric resident males; these results are more similar to those of Neuhaus' (2006) study (70% adult males were philopatric). Given this level of philopatry, one tactic Columbian ground squirrels could use to avoid inbreeding is emigration when close female relatives are present in their natal population. We hypothesized that philopatric male samples would not co-occur with close female relatives in order to avoid inbreeding. Our results supported this hypothesis in regards to co-occurrence with littermate sisters but not in regards to co-occurrence with mothers.

Of the resident male samples, 46.5% co-occurred with one or more close female relative samples; more specifically, 36.6% occurred with their mothers while 28.8% co-occurred with littermate sisters. Regarding mothers, resident males co-occurred with their mother at levels similar to those of females. However, female samples were significantly more likely (1.86 times) to co-occur with one or more littermate sisters. This result is somewhat in contrast to Neuhaus' (2006) conclusion that neither mothers nor littermate sisters affected male emigration. While the presence of only their mother did not cause males to emigrate in large enough numbers to cause a significant disparity in co-occurrence with mother between male and female samples, co-occurrence of adult male samples with one or more littermate sisters should be addressed more closely.

An argument could be made that the presence of the littermate sister resulted in resident males emigrating. This could occur if mothers were aggressive towards their sons if they had daughters present. Lab experiments with Richardson's ground squirrels found that mothers interact more positively with their yearling females, including their daughters, than with male yearlings (Michener and Michener, 1973). Other studies have suggested that parents may benefit by forcing some of their offspring to disperse in order to reduce competition between siblings (Bélichon et al., 1996; Waser et al., 2013). Additionally, Columbian ground squirrel females tend to live in close proximity to their mothers and close female relatives, and occasionally even shift their territory to create space for one another (Arnaud et al., 2012). Neuhaus et al. (2004) found that two-year-old females have increased reproductive success when in female kin clusters. Females also gain significant fitness benefits if closely related kin are in the population with them (Viblanc et al., 2010; Dobson et al., 2012). Due to the findings of this previous research, we suggest that it's possible that females may be aggressive towards sons if daughters are present. Mothers might gain benefits by retaining kin in the population; by encouraging sons to leave if a female also has a daughter, mothers gain those benefits while also minimizing competition between their offspring, and she also minimize the possibility of inbreeding. Our study does not include data on behavioral interactions, therefore our idea that mother aggression towards sons when daughters are present is hypothetical. Future research on sex-biased aggression from mothers may provide enlightening details on the effects of daughters on male emigration and philopatry patterns.

Co-occurrence with mother and male mating

Due to the fact that resident males co-occur with close female relatives in this study, and therefore have the opportunity for inbreeding, we tested our next hypothesis: resident males avoid inbreeding by not mating with any females when their close female relatives are present. Although males were less likely to mate when their mother or littermate sister(s) were present, there were still some males that mated when co-occurring with close female relatives, therefore this hypothesis was not fully supported. We found that male samples were significantly more likely to mate (1.34 times) if they did not co-occur with their mother (91.1% compared to 68.0%, respectively). However, when male samples that co-occurred with their mothers did mate, there was no significant difference in the number of mates or number of offspring produced. These results may be explained by other variables we analyzed. Resident male samples that did not co-occur with mothers and did mate were of similar average weight as resident male samples that co-occurred and mated, and they also emerged from hibernation at approximately the same time, on average. Because emergence dates of these groups of male samples were similar, these males likely had roughly equal access to mates. Had one group emerged later, they would have lost access to mating opportunities with females that had been active prior to their emergence and had already finished estrous (Michener, 1984) These two variables could explain why resident males had similar numbers of mates and offspring produced regardless of whether their mother was present. Ultimately, because the male samples that co-occurred with their mothers had a similar number of mating partners and produced similar number of offspring, males still have the potential of inbreeding with their close female relatives. Age, however, provides an explanation for the finding that a lower proportion of male samples mate when their mother is present.

Male samples that did not co-occur with their mother but did mate were significantly older on average (4.6 years old) than male samples that co-occurred and did not mate (2.0 years old) and male samples that co-occurred and did mate (2.8 years old). Logically, as males get older, they're less likely to co-occur with their mother because she'll be more likely to die. Older males, such as those in the did not co-occur/did mate group, are also more likely to mate, and, importantly, our results showed that while 100% of male samples three-years-old and older mated, only 47.8% of two-year-old male samples mated. Additionally, some of the two-year-olds that didn't mate may not have been able to due to incomplete physiological maturity, as evidenced by the smaller testes observed on some two-year-olds (F.S. Dobson, personal observations). The age differences between male samples that co-occur with their mothers and those that didn't but did mate, and the differences in mating likelihood that result from age, provide a possible explanation for the differences regarding co-occurrence with mother and mating likelihood and why a lower proportion of male samples mate when their mother is present. Alternatively, resident males could wait to mate until their mother is no longer in the population, but the age data in our study is a better supported explanation.

Co-occurrence with sister and male mating

In addition to the decreased likelihood of male samples co-occurring with one or more littermate sisters in comparison to female samples, male samples that did not co-occur with their littermate sisters were 1.45 times more likely to mate than those that did co-occur; this difference was nearly statistically significant. However, similar to co-occurrence with mother, male samples that co-occurred with littermate sisters had no significant differences in number of mating partners and number of offspring produced. These findings can be partly explained by weight and age.

As mentioned above, some Columbian ground squirrels that are particularly lightweight have been noted to emigrate during their two-year-old summer, rather than as yearlings (Festa-Blanchet and King, 1984). Our results similarly showed that younger male samples, particularly two-year-old samples, and lighter male samples are less likely to mate. Male samples that co-occurred with their littermate sisters were significantly younger (1.5 years younger on average) and lighter (81.7 g lighter on average) than male samples that did not co-occur with littermate sisters.

The above, in part, explains the difference in mating probability when co-occurring with littermate sisters. A closer look at age, however, reveals important details. We think that the majority of the male samples that co-occurred with their littermate sisters might have been late dispersers. Of the 11 male samples that co-occurred with one of more littermate sisters, eight individuals (72.7%) were two-year-olds, and six of those eight were no longer in the population by age three. Only two resident males that co-occurred with their littermate sisters and mated remained in the population past age two. Regarding weight, male samples that co-occurred with one or more littermate sister and mated were nearly significantly heavier than male samples that co-occurred but did not mate (485.0±62.1 SD g on average compared to 371.3±43.5 SD g, respectively). A hypothesis previously proposed by other researchers could provide an explanation for the lighter weight observed in male samples that co-occurred with their littermate sisters and didn't mate; the ontogenetic switch hypothesis, in which males disperse when the reach a sufficient size or body fat (stored energy) to do so (Holekamp, 1986; Holekamp, 1984a; Holekamp, 1984b). Wiggett and Boag (1992) argued that body mass did not influence dispersal in their study on Columbian ground squirrels, but the ontogenetic switch hypothesis is worth mentioning, regardless.

Inbreeding

Although resident male samples co-occurring with a close female relative were less likely to mate with one or more females, there were still many instances of it happening. This means there were still opportunities for inbreeding to occur. During the study, there were 28 instances when an adult resident male sample co-occurred with a close female relative. We hypothesized that when adult male residents co-occur with close female relatives that are mating, individuals will be able to recognize their close relatives and avoid mating with them. Our results supported this hypothesis. Of the 28 possible incestuous matings, there was only one instance where the resident male sample mated with his close female relative (his mother). This mating did not result in any offspring, although the female did have offspring with the other male, an immigrant, which with she mated. This is in contrast to the 2200 possible pairs of unrelated (not mother-son or brother-littermate sister) individuals mating, in which 31.8% did mate. During the year when inbreeding occurred, there were only three adult male samples, and only two of those three mated (one resident and one immigrant). These are particularly unusual circumstances, given that usually there's an average of 8.2±3.8 SD male samples mating each year.

In contrast to our results of only a single instance of close inbreeding, other related species do inbreed at higher levels. Hoogland (1992) found that black-tailed prairie dogs regularly inbreed with distant kin; 26% of the matings he observed involved moderate inbreeding. While typically individuals in the species avoid close inbreeding, 9% of observed copulations of females, in contrast to 3.6% in this study, involved extreme inbreeding. The most common type of close relative inbreeding was between half siblings, but there were six instances of fathers copulating with daughters. Despite the inbreeding, Hoogland (1992) found no signs of inbreeding depression. Similarly, yellow-bellied marmots do not avoid inbreeding, and although inbred offspring have a decreased survival rate, males that inbreed have equivalent reproductive success overall compared to males that don't (Olson et al., 2012). In light of these studies, it may be beneficial in future research to measure breeding between extended relatives in Columbian ground squirrels, as well as between fathers and daughters. These potential pairings would be less likely to recognize each other if the species uses social learning to recognize relatives, and this future research would help test the hypotheses regarding the effects of kin recognition on breeding habits in Columbian ground squirrels.

Our results regarding inbreeding provide strong evidence that Columbian ground squirrels are able to recognize and avoid mating with close relatives, perhaps through social learning or scent. Several studies support that female ground-dwelling sciurids, including Columbian ground squirrels, are able to identify kin through scent (Michener and Michener, 1973; Harris and Murie, 1982). Scent may possibly be the mechanism Columbian ground squirrels use to avoid inbreeding. Interestingly, given that the female did not produce offspring in the inbred mating, perhaps the females employed another mechanism to avoid insemination, such as cryptic mate choice. Perhaps removal of the copulatory plug or ejection of the sperm could enable Columbian ground squirrel females to prevent fertilization in the rare event that it occurs (Raveh et al, 2011). Our final hypothesis was that, in the event resident males mated with close female relatives, they would not produce any offspring due to either natural abortion from inbreeding depression or due to cryptic female choice. There are so few instances of close inbreeding in this study that we can neither support nor reject this hypothesis.

An area that our inbreeding analysis does not address is attempted matings. This analysis only includes matings that either did or did not occur via underground courtships. In the future, to better understand tactics used to avoid inbreeding in Columbian ground squirrels, behavioral interactions between adult resident males and their close female relatives could be monitored to observe whether resident males attempt to mate with their female relatives or vice versa.

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Tables and Figures

Table 1.1. Pearson's chi-squared with Yates' continuity for correction of resident adult male co-occurrence with their mother with observed counts of individuals, expected counts of individuals in parentheses, and standardized residuals (Pearson's χ^2 =2.633, d.f.=1, N=311, p=0.105).

	Females	Males	Total Observed
Co-occurred			
Obs/Exp	62 (67.9)	26 (20.1)	88
Residual	-0.72	1.32	
Did not co-occur			
Obs/Exp	178 (172.1)	45 (50.9)	223
Residual	0.45	-0.83	
Total Observed	240	71	311

Table 1.2. Pearson's chi-squared with Yates' continuity for correction of resident adult male co-occurrence with one or more of their littermate sisters with observed counts of individuals, expected counts of individuals in parentheses, and standardized residuals (Pearson's χ^2=4.3699, d.f.=1, N=311, p=0.037, φ=0.119).

	Females	Males	Total Observed
Co-occurred			
Obs/Exp	69 (61.7)	11 (18.3)	80
Residual	0.92	-1.70	
Did not co-occur			
Obs/Exp	171 (178.2)	60 (52.7)	231
Residual	-0.54	1.00	
Total Observed	240	71	311

Table 1.3. Fisher's exact test of resident adult male co-occurrence with their mother and whether each male mated with one or more females. Included are observed counts of individuals, expected counts of individuals in parentheses, and standardized residuals (d.f.=1, N=70, 95% CI [1.09, 24.32], p=0.021).

		Did not co-occur with mother	Co-occurred with mother	Total Observed
Mated				
	Obs/Exp	41 (37.3)	17 (20.7)	58
	Residual	0.61	-0.81	
Did not mate				
	Obs/Exp	4 (7.7)	8 (4.3)	12
	Residual	-1.34	1.79	
Total Observed		45	25	70

Table 1.4. ANOVA with post-hoc Tukey's HSD test comparisons of average ages co-occurrence with mother/mating groups of adult resident males (d.f.=3, F=12.57, p<0.001). The top row of each comparison indicates the groups compared and the average difference between the two, followed by the 95% confidence interval and p-value of the comparison.

	Co-occurred, Mated (1) n=17	Co-occurred, Did not mate (2) n=8	Did not co-occur, Mated (3) n=41
Did not co-occur, Did not mate (4) n=4	-0.8 years (4-1) 95% CI [-2.92, 1.39] p=0.786	0.0 years (4-2) 95% CI [-2.38, 2.38] p=1.000	-2.6 years (3-4) 95% CI [-4.62, -0.55] p=0.007
Did not co-occur, Mated (3) n=41	1.8 years (3-1) 95% CI [0.70, 2.94] p<0.001	2.6 years (3-2) 95% CI [1.09, 4.09] p<0.001	-
Co-occurred, Did not mate (2) n=8	-0.8 years (2-1) 95% CI [-2.43, 0.90] p=0.622	-	-

Table 1.5. ANOVA with post-hoc Tukey's HSD test comparisons of average mass of co-occurrence with mother/mating groups of adult resident males (left portion of table; d.f.=3, F=10.57, p<0.001) and average number of days adult resident males emerged post emergence of first male of that year (right portion of the table; d.f.=3, F=18.37, p<0.001). The top row of each comparison indicates the groups compared and the average difference between the two, followed by the 95% confidence interval and p-value of the comparison.

	Co-occurred, Mated (1) n=17	Co-occurred, Did not mate (2) n=8	Did not co-occur, Mated (3) n=41	Did not co-occur, Did not mate (4) n=4
Did not co-occur, Did not mate (4) n=4	-98.38 g (4-1) 95% CI [-198.49, 1.73] p=0.558	-36.25 g (4-2) 95% CI [-146.57, 74.07] p=0.822	-146.65 g (4-3) 95% CI [-241.01, -52.28] p<0.001	-
Did not co-occur, Mated (3) n=41	48.26 g (3-1) 95% CI [-3.70, 100.23] p=0.078	110.40 g (3-2) 95% CI [40.77, 180.02] p<0.001	-	18.5 days 95% CI [10.19, 26.76] p<0.001
Co-occurred, Did not mate (2) n=8	-62.13 g (2-1) 95% CI [-139.37, 15.10] p=0.157	-	-11.2 days (3-2) 95% CI [-17.34, -5.11] p<0.001	7.3 days (4-2) 95% CI [-2.43, 16.93] p=0.208
Co-occurred, Mated (1) n=17	-	11.1 days (2-1) 95% CI [4.35, 17.91] p<0.001	-0.1 days (3-1) 95% CI [-4.65, 4.47] p=0.999	18.4 days (4-1) 95% CI [9.60, 27.17] p<0.001

Table 1.6. Fisher's exact test of resident, adult male co-occurrence with one or more littermate sisters and whether each male mated with one or more females. Included are observed counts of individuals, expected counts of individuals in parentheses, and standardized residuals (d.f.=1, N=70, 95% CI [0.71, 23.00], p=0.061).

		Did not co-occur with littermate sister	Co-occurred with littermate sister	Total Observed
Mated				
	Obs/Exp	52 (49.7)	6 (8.3)	58
	Residual	0.32	-0.79	
Did not mate				
	Obs/Exp	8 (10.3)	4 (1.7)	12
	Residual	-0.71	1.75	
Total Observed		60	10	70

Table 1.7. ANOVA with post-hoc Tukey's HSD test comparisons of average mass of co-occurrence with littermate sister(s)/mating groups of adult resident males (d.f.=3, F=9.71, p<0.001). The top row of each comparison indicates the groups compared and the average difference between the two, followed by the 95% confidence interval and p-value of the comparison.

	Co-occurred, Mated (1) n=6	Co-occurred, Did not mate (2) n=4	Did not co-occur, Mated (3) n=52
Did not co-occur, Did not mate (4) n=8	-45.63 g (4-1) 95% CI [-144.23, 52.98] p=0.617	68.13 g (4-2) 95% CI [-43.69, 179.94] p=0.382	-90.24 g (3-4) 95% CI [-159.58, -20.90] p=0.006
Did not co-occur, Mated (3) n=52	44.62 g (3-1) 95% CI [-34.11, 123.34] p=0.447	158.37 g (3-2) 95% CI [63.62, 253.11] p<0.001	-
Co-occurred, Did not mate (2) n=4	-113.75 g (2-1) 95% CI [-231.61, 4.11] p=0.062	-	-

Table 1.8. Pearson's chi-squared test with Yates' continuity for correction of the relatedness of all possible mating pairs of adults from 2005-2012 and 2014-2015 with observed instances of mating, expected instances of mating in parentheses, and standardized residuals (Pearson's χ^2 =4.526, d.f.=1, N=2228, p=0.003).

		Unrelated pair	Related Pair	Total Observed
Mated				
	Obs/Exp	700 (692.2)	1 (8.8)	701
	Residual	0.3	-2.63	
Did not mate				
	Obs/Exp	1500 (1507.8)	27 (19.2)	1527
	Residual	-0.20	1.78	
Total Observed		2200	28	2228

Table 1.9. Probability of mating for each sample of pairs of related individuals for 2005-2015. Also included is the year the pair was present, the left and right ear tags of each individual, the relationship of the pair indicated next to the female (m for mother-son and s for littermate sister-littermate brother), and their observed mating status. All males two years and older and all mating females are included. Unknown is listed for probability in 2013 due to missing mating logs. Unknown is listed for "mated" if the female's mating log was not recorded.

Year	Male	Female relative	Chance of mating (%)	Mated
2006	b350-a593	2801-3445 (m)	28.0	No
2006	a573-a573	b384-3583 (m)	27.3	No
2006	b323-b323	b321-b321 (s)	27.8	No
2007	b345-b345	b348-b348 (s)	20.5	No
2007	b345-b345	b346-b346 (s)	19.7	No
2007	b337-b337	2801-3445 (m)	19.9	No
2007	c935-a593	2801-3445 (m)	20.4	No
2007	b376-b376	2823-2823 (m)	20.5	No
2007	b337-b337	b335-b335 (s)	20.8	Unknown
2007	b360-b376	b365-b365 (s)	20.5	No
2007	b362-b362	b365-b365 (s)	19.9	No
2008	b345-b345	b348-b348 (s)	33.4	No
2008	b345-b345	b346-b346 (s)	33.1	No
2008	b366-b366	b319-b319 (m)	33.9	Unknown
2008	c904-c275	b317-b318 (m)	33.7	No
2009	b366-b366	c278-b319 (m)	25.5	No
2009	c956-c956	b346-b346 (m)	24.9	No
2009	b345-b345	b346-b346 (s)	24.9	No
2009	b345-b345	b348-b348 (s)	25.4	No
2009	c971-c971	b369-b369 (m)	26.0	No
2009	c904-c275	b317-b318 (m)	25.6	Unknown
2010	c971-c971	b369-b369 (m)	33.2	Yes
2012	d019-d019	d021-d021 (s)	27.1	Unknown
2012	d048-d048	b340-b340 (m)	26.6	No
2012	d030-d030	d432-d432 (m)	26.2	No
2012	d019-d019	c972-c972 (m)	26.6	No
2013	e114-e114	c282-d098 (m)	Unknown	Unknown
2013	e113-e113	c282-d098 (m)	Unknown	Unknown
2013	e115-e115	c282-d098 (m)	Unknown	Unknown
2013	e124-e124	e123-e123 (s)	Unknown	Unknown
2013	e124-e124	d432-d432 (m)	Unknown	Unknown
2013	d019-d019	d021-d021 (s)	Unknown	Unknown
2014	e537-e537	d043-d043 (m)	21.3	No
2014	e114-e635	d098-c282 (m)	21.2	No
2014	e124-e124	d432-d432 (m)	21.1	Unknown
2015	d900-e635	d098-c282 (m)	29.8	No
2015	d894-e753	e142-e142 (m)	29.5	No
2015	e124-e124	d432-d432 (m)	28.1	No
2015	d896-e537	d043-d043 (m)	28.9	Unknown
2015	d894-e753	e754-e754 (s)	29.4	No

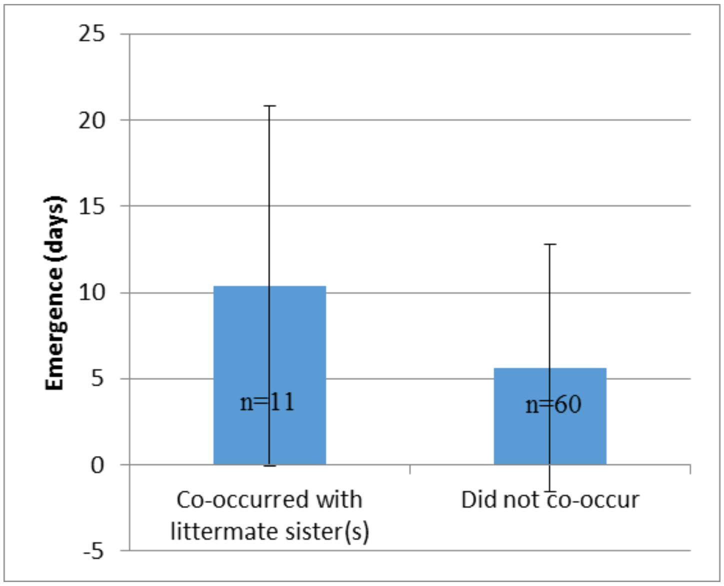

Figure 1.1. Average number of days resident adult males emerged after first squirrel of that season emerged, with standard deviation, based on whether they co-occurred with one or more littermate sisters (Welch two sample t-test: d.f.=11.81, 95% CI [-11.91, 2.42], p=0.174).

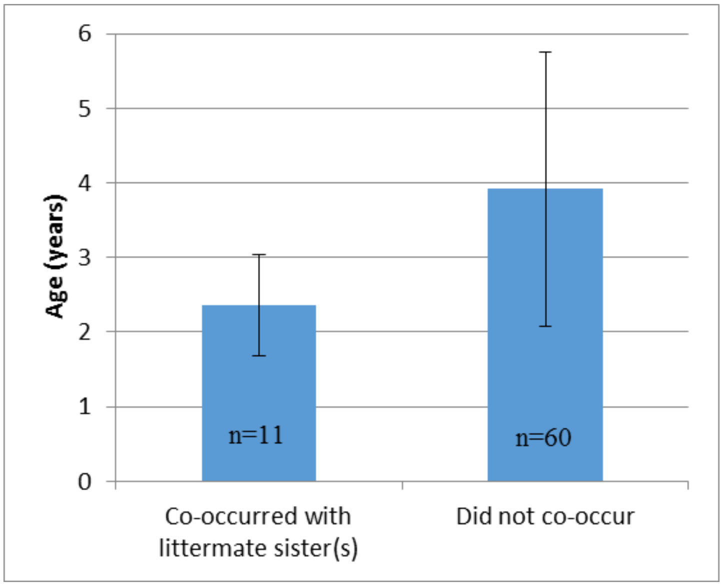

Figure 1.2. Average ages of resident adult males, with standard deviation, based on whether they co-occurred with one or more littermate sisters (Welch two sample t-test: d.f.=42.64, 95% CI [0.92, 2.36], p<0.001).

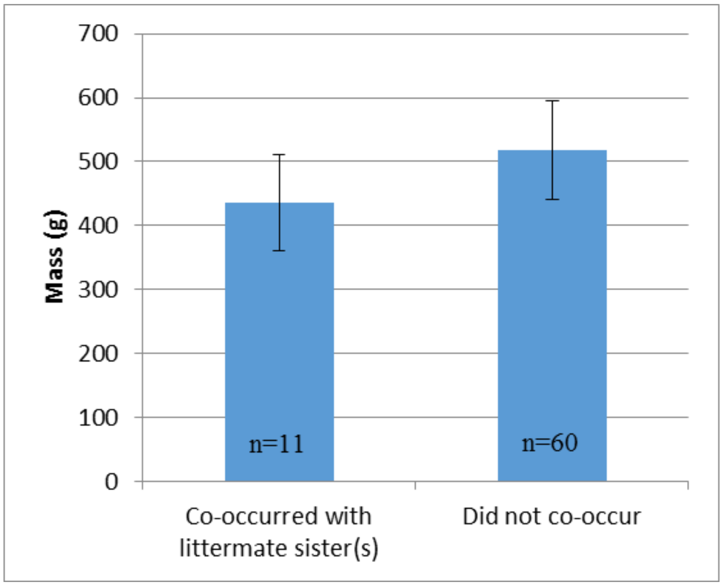

Figure 1.3. Average weight of resident adult males, with standard deviation, based on whether they co-occurred with one or more littermate sisters (Welch two sample t-test: d.f.=14.04, 95% CI [28.26, 135.09], p=0.005).

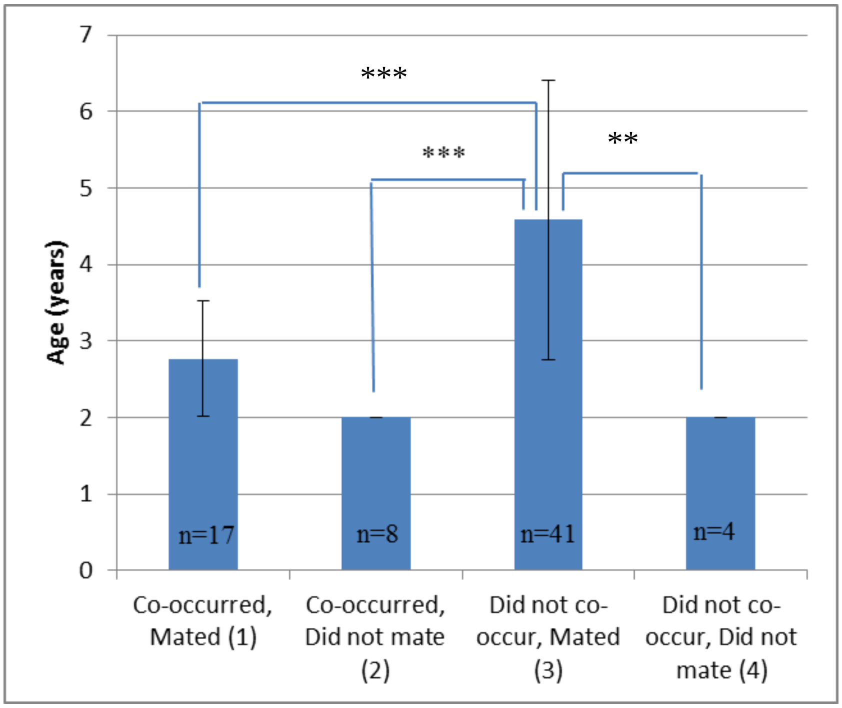

Figure 1.4. Average age, with standard deviation, of adult resident males in the four co-occurrence with mother-mating categories based on whether the male co-occurred with his mother that year and if the male mated with one or more females that year (ANOVA: d.f.=3, F=12.57, p<0.001).
Note: ** significance at the 0.01 level; *** significance at the 0.001 level.

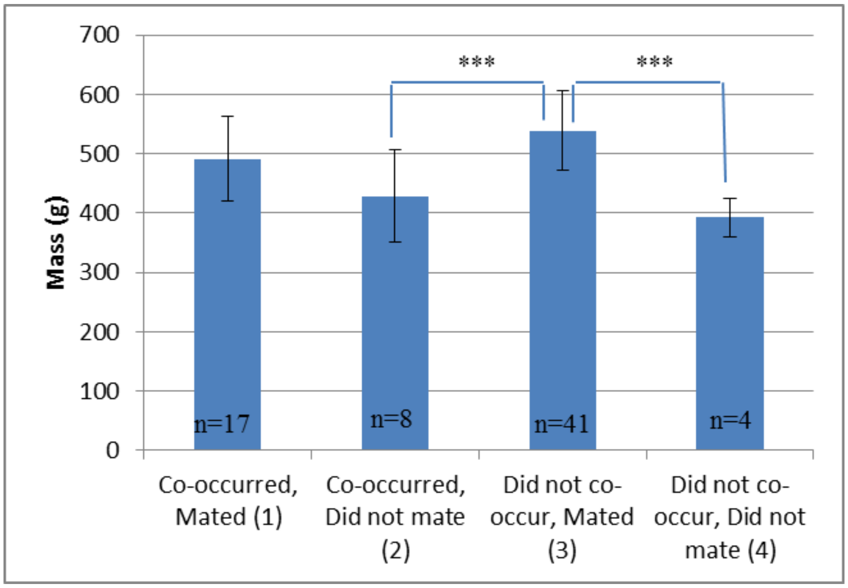

Figure 1.5. Average mass, with standard deviation, of adult resident males in the four co-occurrence with mother-mating categories based on whether the male co-occurred with his mother that year and if the male mated with one or more females that year (ANOVA: d.f.=3, F=10.57, p<0.001).

Note: *** significance at the 0.001 level.

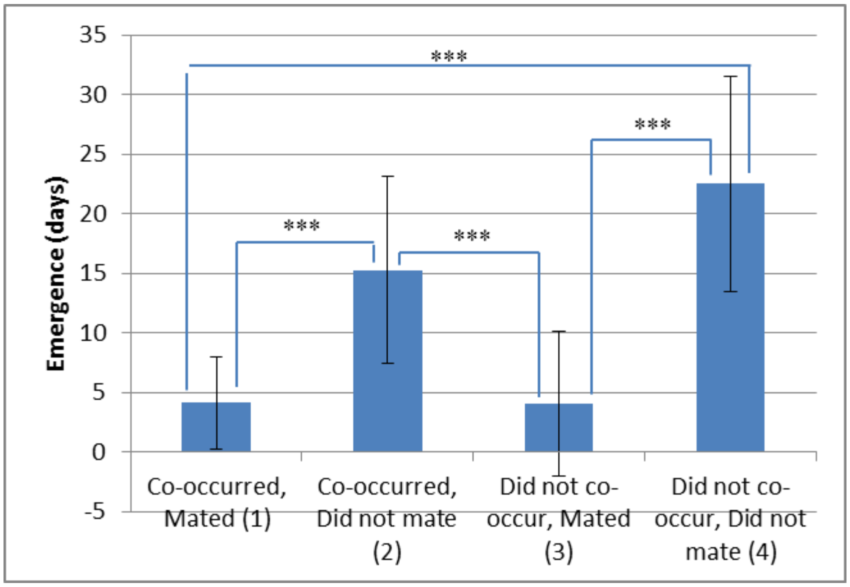

Figure 1.6. Average number of days emerged past the emergence date of the first squirrel of that year, with standard deviation, of adult resident males in the four co-occurrence with mother-mating categories based on whether the male co-occurred with his mother that year and if the male mated that year (ANOVA: d.f.=3, F=18.37, p<0.001). Sample sizes: category 1, n=4; category 2, n=8; category 3, n=41; category 4, n=4)

Note: *** significance at the 0.001 level.

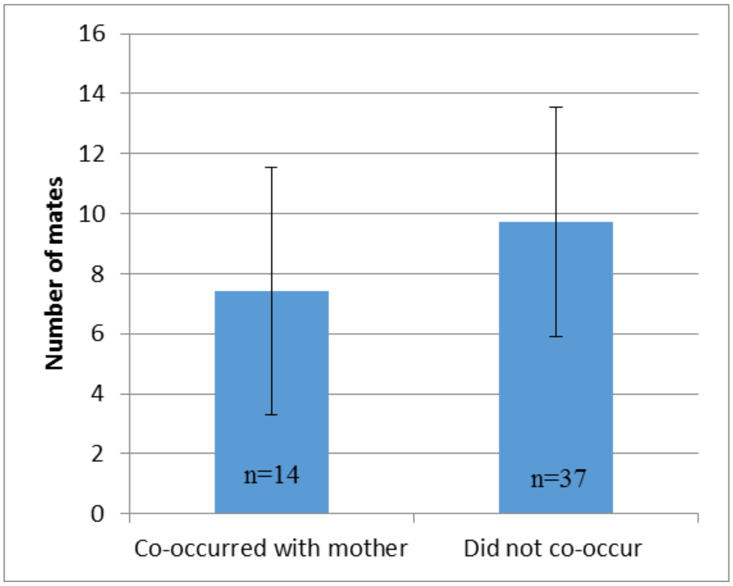

Figure 1.7. Average number of mates of resident, adult males that mated, with standard deviation, based on whether they co-occurred with their mother (Welch two sample t-test: d.f.=22.03, 95% CI [-4.94, 0.33], p=0.084).

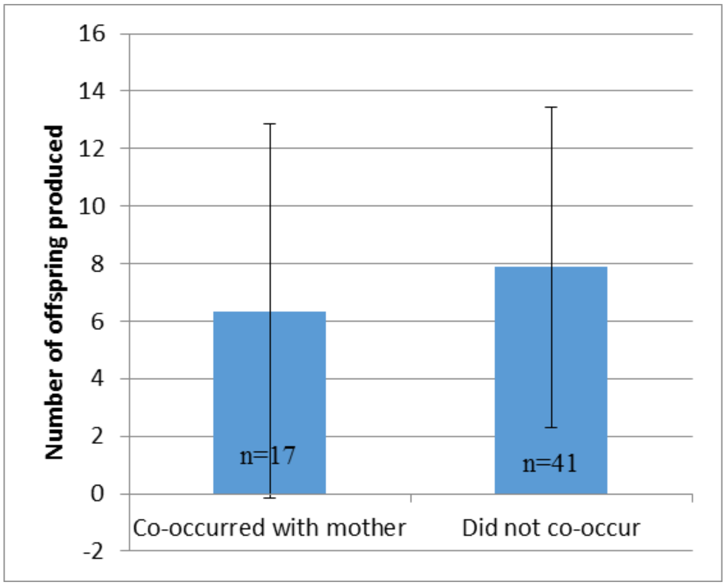

Figure 1.8. Average number of offspring of resident adult males that mated, with standard deviation, based on whether they co-occurred with their mother (Welch two sample t-test: d.f.=26.19, 95% CI [-5.24, 2.19], p=0.406).

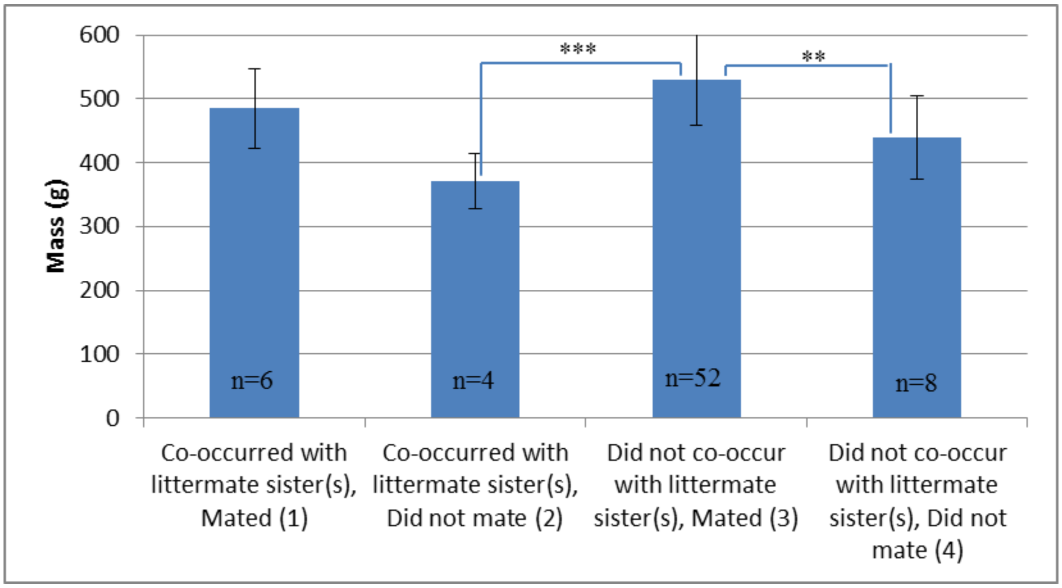

Figure 1.9. Average mass, with standard deviation, of adult resident males in the four co-occurrence with littermate sister-mating categories based on whether the male co-occurred with one or more littermate sisters that year and if the male mated with one or more females that year (ANOVA: d.f.=3, F=9.71, p<0.001).
Note: ** significance at the 0.01 level; *** significance at the 0.001 level.

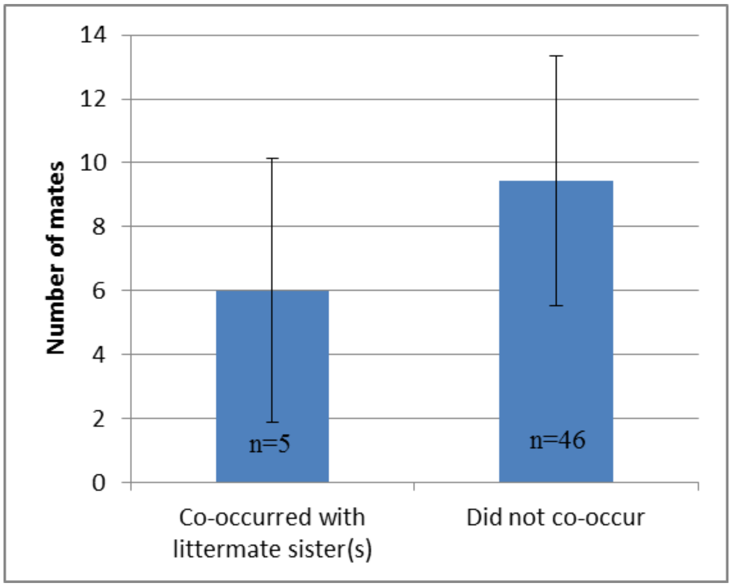

Figure 1.10. Average number of mates of resident adult males that mated, with standard deviation, based on whether they co-occurred with one or more littermate sisters (Welch two sample t-test: d.f.=4.81, 95% CI [-8.46, 1.59], p=0.138).

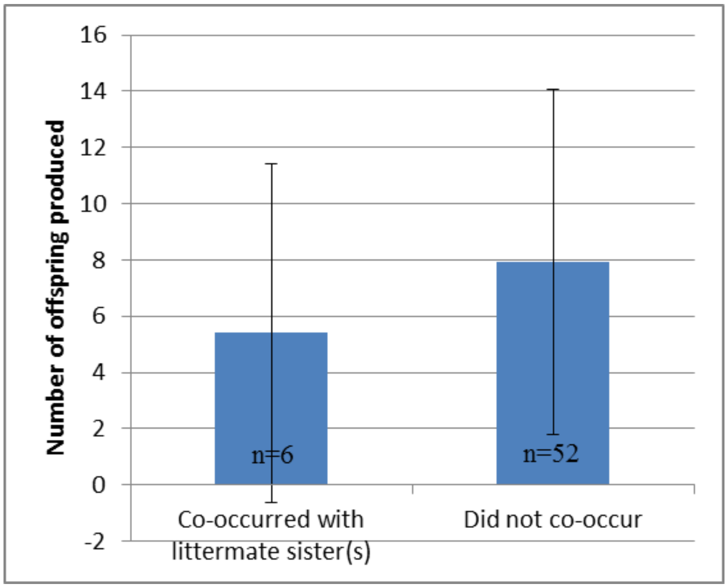

Figure 1.11. Average number of offspring of resident adult males that mated, with standard deviation, based on whether they co-occurred with one or more littermate sisters (Welch two sample t-test: d.f.=6.23, 95% CI [-7.35, 4.90], p=0.644).

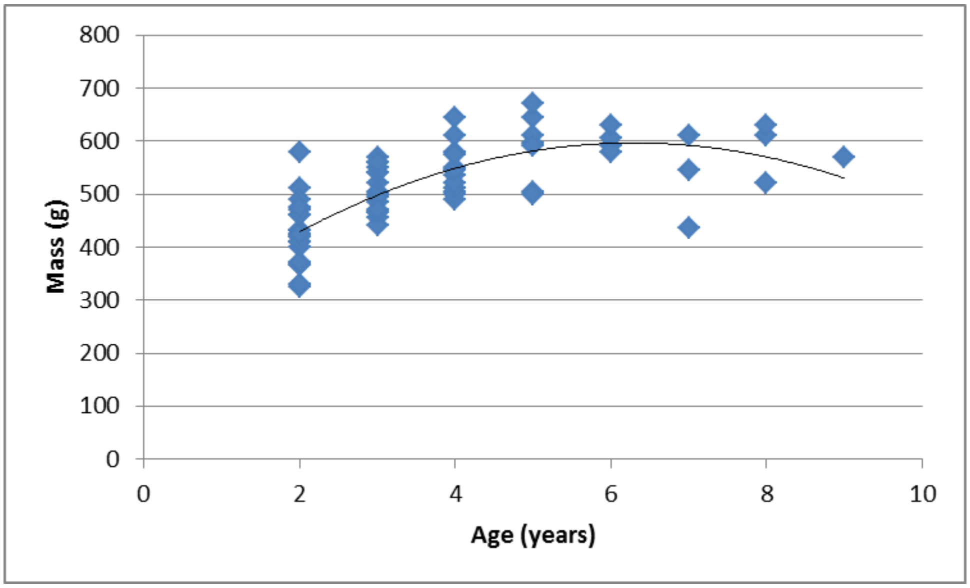

Figure 1.12. Relationship between age and mass in adult resident males (r^2=0.57, d.f.=68, p<0.001).

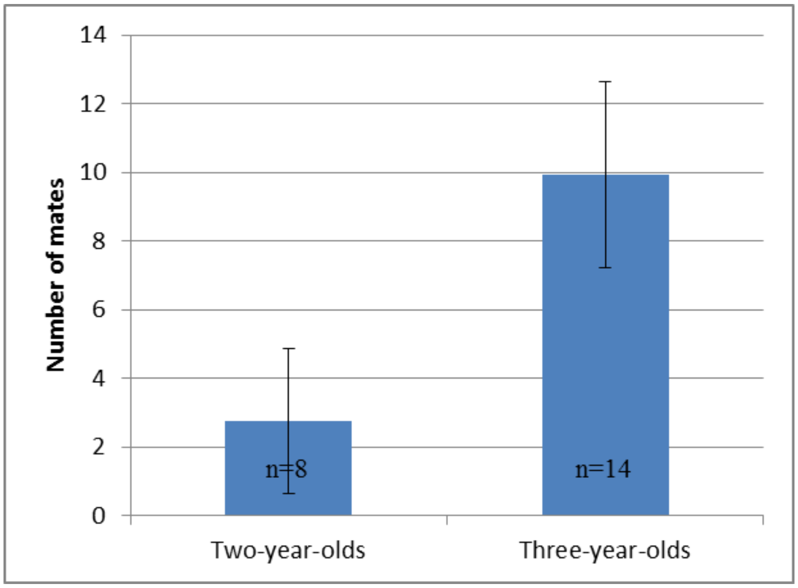

Figure 1.13. Average number of mates of two-year-old resident males (including only males that mated with one or more female) compared to three-year-old resident males, with standard deviation (d.f.=17.77, 95% CI [-9.37, -4.99], p<0.001).

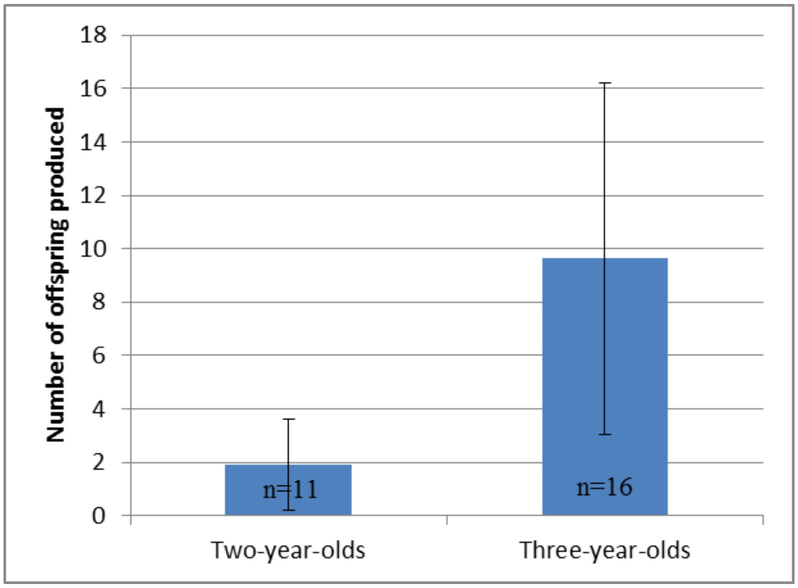

Figure 1.14. Average number of offspring of two-year-old resident males (including only males that mated with one or more female) compared to three-year-old resident males, with standard deviation (d.f.=17.82, 95% CI [-11.329, -4.103], p<0.001).

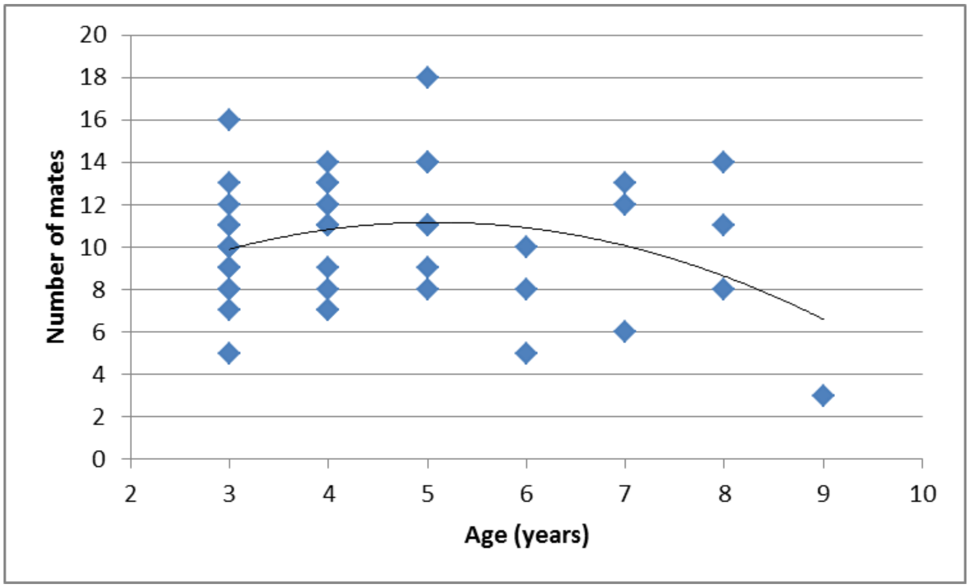

Figure 1.15. Relationship between age and number of mates for resident males aged three years old and older (r^2=0.04, d.f.=40, p=0.160).

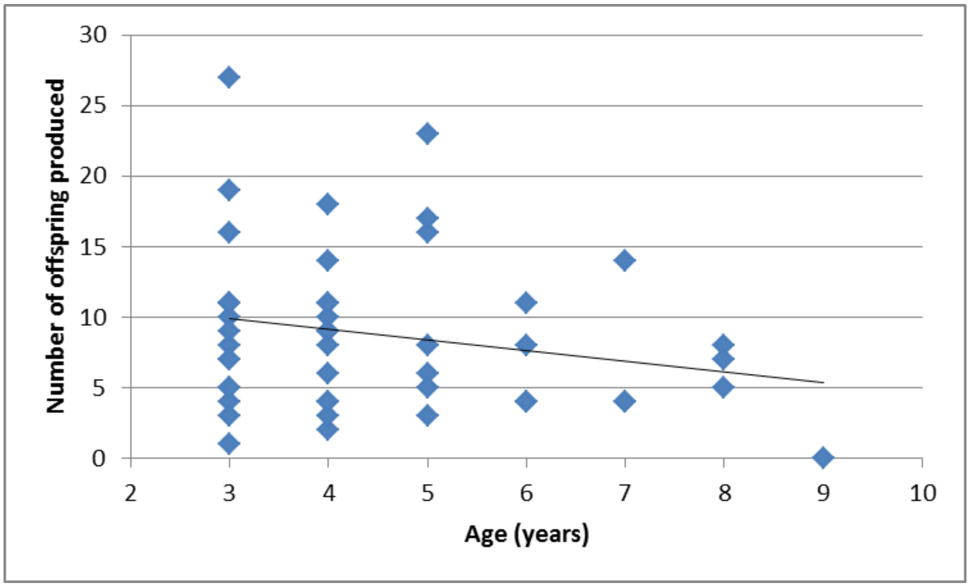

Figure 1.16. Relationship between age and number of offspring produced for resident males aged three years and older (r^2=0.03, d.f.=45, p=0.145).

Chapter II

The effects of residency status on fitness in Columbian ground squirrel males

Abstract

In many species, inbreeding can have detrimental consequences on fitness. A method of avoiding inbreeding is through dispersal from the natal population, but dispersal may be costly and dangerous. The goal of our study was to determine if residency status (immigrant compared to philopatric resident) affects fitness of males in the polygynandrous Columbian ground squirrel. We proposed a variety of hypotheses to explain how annual reproductive success varied with residency status and whether specific attributes of male circumstances (age, date of emergence from hibernation, length of time in the population, and mass) contributed to annual fitness. Resident adult male samples were 1.28 times more likely to mate than immigrant male samples, and resident male samples had significantly more mating partners on average (7.4±5.1 SD mates compared to 4.7±5.1 SD mates; d.f.=105.28, CI [-4.54, -0.71], p=0.008). We concluded this is a result of the influences of age and date of emergence from hibernation. Although number of mates was not influenced by age after age two, that difference at age two may partly explain the difference in mating probability of adult resident and immigrant male samples. Two-year-old resident male samples were 4.31 times more likely to mate than two-year-old immigrant samples, and adult resident male samples were nearly significantly older, on average, than immigrant male samples (3.7±1.8 years and 3.2±1.2 years, respectively; d.f.=121.58, 95% CI [-1.03, 0.03], p=0.06). Emergence date was significantly related to number of mates, in which each day later a male emerged, he had a 0.8 mate (±0.25; ±95% CI) decrease

in number of mating partners (r^2=0.51, d.f.=108, p<0.001) with a significant polynomic curve of a 0.01 mate [±0.01; ±95% CI] increase in mating partners beginning around emergence day 25 (p=0.002). Resident adult male samples emerged (or, in the case of first-year immigrants, “appeared”) significantly earlier, on average, compared to immigrant male samples (6.4±7.9 SD days post first squirrel’s emergence of season compared to 11.2±10.1 SD days; d.f.=101.90, 95% CI [-8.13, -1.60], p=0.004). Emergence date is also significantly related to age, in which every one-year increase in age results in a 10.8 day (±4.0; ±95% CI) decrease in emergence date (i.e. emerged earlier; r^2=0.34, d.f.=68, p<0.001) for resident male samples and a 15.8 day (±11.95; ±95% CI) decrease in emergence date (r^2=0.27, d.f.=53, p=0.011) for immigrant male samples, with an increased polynomial curve beginning at age 5 years for both residencies. Resident adult male samples were nearly significantly older, on average age, compared to immigrant male samples (3.7±1.8 years compared to 3.2±1.2 years, respectively; d.f.=121.58, 95% CI [-1.03, 0.03], p=0.06). Age contributed to the earlier average emergence date of adult resident male samples, and the average earlier emergence date contributed to the higher number of mating partners in resident male samples. Despite more mating partners, adult resident male samples and adult immigrant male samples had no significant difference in number of offspring produced (6.1±6.0 SD offspring and 5.0±6.8 SD offspring, respectively; d.f.=111.05, CI [-3.37, 1.20], p=0.348). We suggest that one possible reason resident male samples mated with more females on average but did not have more offspring on average may be inbreeding depression. Immigrant male samples had a nearly significant trend of producing more offspring per mate compared to resident male samples (interaction between residency status and number of mates in regards to number of offspring produced: d.f.=109, p=0.092). For adult male resident samples, for every one mate increase there was a 0.9 offspring (±0.22; ±95% CI) increase in number of offspring produced (r^2=0.50, d.f.=61, p<0.001), but for adult immigrant male samples, for every one mate increase there was a 1.1 offspring (±0.22; ±95% CI) increase in number of offspring produced (r^2=0.69, d.f.=48, p<0.001).

Introduction

Inbreeding: consequences and avoidance

Inbreeding depression, or a reduced fitness of offspring resulting from mating between close relatives, has been a topic of much interest and research for scientists in recent years (Hendrick and Kalinowski, 2000; Pusey and Wolf, 1996). It is particularly applicable in the conservation and wildlife management communities, which use the findings of dispersal and inbreeding research studies to protect vulnerable species (Hendrick and Kalinowski, 2000). When species become threatened or endangered, the chances of inbreeding amongst individuals increases as unrelated mating partners decrease.

Through inbreeding, genetic variation is lost in populations (Allendorf and Leary, 1986). Repetitive breeding between relatives decreases genetic recombination and can result in homozygosity and fixation for deleterious recessive alleles and the consistent expression of that disadvantageous phenotype (Wright, 1969; Pusey and Wolf, 1996; Allendorf and Leary, 1986). Loss of genetic variation can have far reaching fitness consequences for a population and can be seen at an individual level as effects such as sperm deformation, sterility, decreased mating opportunities, reduced reproductive rates, decreased survival, and developmental abnormalities, such as decreased growth rates (Pusey and Wolf, 1996; Allendorf and Leary, 1986). Additionally, if inbreeding is expansive in a population, increased susceptibility to diseases and parasites can also occur (Allendorf and Leary, 1986). Luckily, traits that prevent inbreeding have evolved, including: recognition of relatives; attraction to genetically dissimilar mates;

polygyny, polyandry, and extra-pair mating; cryptic female choice; single sex dispersal; and dispersal prior to sexual maturity.

Many small mammals are able to recognize relatives through methods such as social learning and scent (Lawson Handley and Perrin, 2007). Black-tailed prairie dogs (Hoogland, 1992; Dobson et al., 1997), banner-tailed kangaroo rats (Waser et al., 2012), and Columbian ground squirrels (King, 1989) are able to recognize close kin through social learning; they learn that individuals they grew up with, particularly littermates and mothers, are kin, and as adults they avoid or refuse to mate with them. Through lab experiments and experiments with scent cubes, researchers have supported that Columbian ground squirrels (Harris and Murie, 1982), Richardson’s ground squirrels (Michener and Michener, 1973), meerkats (Leclaire et al., 2012; Harris and Murie, 1982), and Belding’s ground squirrels (Mateo, 2006; Harris and Murie, 1982) are all able to recognize familiar and unfamiliar individuals through scent. One suggested mechanism for how scent recognition occurs is from Mateo (2006); interactions between by-products from the major histocompatibility complex (MHC) and bacteria on gland surfaces and the gastrointestinal tract may create a unique, recognizable odor for each individual. Because the MHC involves so many genes, each individual is unlikely to share a complete genotype, but related individuals are likely to share a large number of genes. Individuals may be able to detect relatedness by comparing how similar their scent is to another’s. They can then select a mate that smells as though they have a genetically dissimilar MHC haplotype in order to avoid inbreeding (Mays and Hill, 2004). Research supports that female preference for opposite-typed MHC haplotyped also occurs in humans (Wedekind et al., 1995). Another possible scent recognition method in rodents is proteins in urine, known as major urinary proteins (MUPs) and alpha 2u globulins (A2Us) (Beynon and Hurst, 2004). These proteins, which bind to pheromones, are products of several discrete genes. As a result, similar to MHC byproducts, a wide range of haplotypes exist, and MUPs may also act as a signal of relatedness.

In the event that individuals do mate with close relatives, mating with multiple partners may decrease the chances of producing inbred offspring. Mating with multiple individuals, either through polyandry or extra-pair mating, provides a female with the opportunity to still produce offspring if she is genetically incompatible with one mate (Zeh and Zeh, 2001). If multiple paternity occurs in the litter, such as occurs in polygynandrous Columbian ground squirrels, individuals will also increase their chances of producing at least some offspring with genetic variability, even if she does mate with some related males (Murie, 1995; Raveh et al., 2011). Another possible, yet controversial, mechanism for preventing inbreeding post-mating is cryptic mate choice, in which a female “chooses” the sperm of one male over another (Birkhead, 1998). This could occur through sperm ejection (Birkhead, 1998; Birkhead, 2010). Another method of choosing sperm is through removal of the post-mating copulatory plug, which, when present, blocks the sperm of other males; this method occurs in Columbian ground squirrels (Murie and McLean, 1980; Raveh et al., 2011). Differential abortion of embryos, a mostly theoretical idea, could be another tactic of minimizing inbred offspring (Birkhead, 1998).

Some species can prevent the possibility of inbreeding ever occurring through sex-biased and age-biased dispersal (Lawson Handley and Perrin, 2007). If one sex, typically the males in mammal species, disperses further than the other when young, particularly prior to sexual maturity, then the likelihood of related individuals ever mating is greatly decreased (Pusey, 1987; Greenwood, 1980). Columbian ground squirrels are one example of juvenile male-biased dispersal (Festa-Blanchet and King, 1984; Wiggett and Boag, 1989; Neuhaus, 2006). In mammals, such as Columbian ground squirrels, females have a higher parental investment, and as a result, benefit by remaining philopatric and maintaining a familiar environment to raise her offspring (Pusey, 1987; Greenwood, 1980; Murie and Harris, 1984). Alternatively, males benefit by mating with as many females as possible, and dispersal may increase their chances of finding more mates, particularly those with better genetic compatibility.

Benefits of dispersal and outbreeding

Dispersal has a variety of benefits, particularly those related to individual fitness and health and overall population stability. Researchers have proposed a variety of explanations for how dispersal evolved, and no single hypothesis fully explains the evolution for all species (Dobson and Jones, 1985; Greenwood, 1980). One explanation is dispersal could have evolved as a by-product of natural selection for males that had high levels of activity that increased their access to mates as they wandered between populations (Holekamp and Sherman, 1989; Lidicker, 1962). Problematically, several studies, such as Waser et al.'s (2013) study on banner-tailed kangaroo rats, support that males that disperse actually have equivalent, rather than increased, access to mates. Belding's ground squirrel males were found to have similar access to mates at their dispersal site as they would have had at their natal site (Holekamp and Sherman, 1989). Similarly, Columbian ground squirrel immigrants have an equivalent number of mates as philopatric residents when only the first mates of each female are analyzed (Neuhaus, 2006). Related to accessing more mates by dispersing, competition for mates, particularly competition with kin for mates, encourages dispersal, especially in polygynous and promiscuous species, such as banner-tailed kangaroo rats (Dobson, 1982; Waser et al., 2013). This hypothesis is not entirely supported either, however. For instance, adult California ground squirrels were hypothesized to be aggressive towards juvenile males to encourage dispersal and minimize competition for mates, but Dobson (1979) found, through field observations, that this was not supported.

Another hypothesis is that dispersal results from physiological traits and processes, not social interactions as described above. Specifically the achievement of a certain weight or fat store level in some species, particularly ground-dwelling scuirids, may cause dispersal (Holekamp, 1984a). This hypothesis was supported for Belding’s ground squirrels (Holekamp, 1984b; Holekamp and Sherman, 1989; Holekamp, 1986) but not Columbian ground squirrels (Wiggett and Boag, 1992). In Belding’s ground squirrels, when a certain body mass is reached, behavioral changes occur, including increased exploration and locomotion and decreased fear response (Holekamp, 1986).

A particularly popular explanation for the evolution of dispersal is inbreeding avoidance (Pusey and Wolf, 1996; Holekamp, 1984a; Lawson Handley and Perrin, 2007). The benefit of inbreeding avoidance, regardless of whether it occurs through dispersal or another one of the mechanisms described above, is minimization of inbreeding depression and fixation of deleterious alleles and maximization of genetic variation and recombination (Pusey and Wolf, 1996; Allendorf and Leary, 1986). Individuals that disperse will be more likely to have offspring with higher fitness, because those offspring may have higher heterozygosity and the possibility of creating new, advantageous genetic recombinations (Lidicker, 1962). Males with high heterozygosity, which have increased resistance to diseases and pathogens, may be preferred as mates by females due to their increased health (Allendorf and Leary, 1986; Mays and Hill, 2004). Another way a male could be particularly attractive to a female is through high mito-nuclear co-adaptation. Genetic recombination, which best occurs through outcrossed mating, helps to maintain mito-nuclear compatibility within an individual, and this compatibility is essential for efficient mitochondrial functioning and energy production (Havird et al., 2015). Mitochondrial DNA mutates quickly; for an individual to have optimal mitochondrial functioning, nuclear DNA must be adapted to those mutations. Genetic recombination and mutations, which outbreeding encourages, aid with mito-nuclear adaptation. A healthy individual, with good genes and mito-nuclear compatibility, may live longer, be preferred by females, and have stronger offspring that inherit their genes (Mays and Hill, 2004). One meta-analysis supports that natal dispersal is associated with higher fecundity in mammalian species (Bélichon et al., 1996). Yellow-bellied marmots don't avoid inbreeding, and inbred offspring have decreased survival (Olsen et al., 2012).

Beyond the genetic benefits of outcrossing and the possible mating and fitness benefits described above, dispersal may have environmental benefits, including increased access to resources, decreased aggression from conspecifics, and reduced kin competition (Lawson Handley and Perrin, 2007). These benefits seem to be skewed towards the females, however, as will be supported in the examples below. Increased access to resources can be an enticement for dispersers, particularly during periods of high population density (Lawson Handley and Perrin, 2007). For example, in an experimental supplemental food study, Dobson (1979) found that in California ground squirrels, females were attracted to adjacent colonies where supplemental food was added. Low population density in areas surrounding their natal colony also seemed to attract them and encourage their dispersal. In North American red squirrels, a fraction of females disperse post-weaning, but females are less likely to disperse if they have food stores left over from the fall (Berteaux and Boutin, 2000). In a different supplemental food experiment with round-tailed ground squirrels, Dunford (1977) found that, although poor food supply increased the tendency for males to disperse, that increase was not statistically significant. Similarly, food availability was not found to influence dispersal in yellow-bellied marmot yearling males or Belding's ground squirrel juvenile males (Holekamp, 1984a).

Although logically territory and nest/hibernation site availability may be a benefit to dispersal, some research has suggested that dispersal may either be unrelated to space availability or may not actually result in higher quality space. For example, in North American red squirrels, females that disperse post-weaning do not gain a higher quality environment (Berteaux and Boutin, 2000). Similarly, banner-tailed kangaroo rat dispersers do not gain access to superior habitat compared to their non-dispersing counterparts (Waser et al., 2013). In yellow-bellied marmots, natal dispersal occurs even when there are excessive burrows (Armitage and Downhower, 1974), and similarly, in black-trailed prairie dogs, dispersal occurs even when parents vacate their nest site (Holekamp, 1984a).

Another theoretical benefit to dispersal is a reduction of aggression from conspecifics, particularly for young males, and competition from relatives (Lawson Handley and Perrin, 2007). Meta-analysis research of ground-dwelling sciurids, however, has indicated that adult aggression is not what drives emigration in males (Holekamp, 1984a). This is supported in California ground squirrels, in which adult males are not particularly aggressive towards younger resident males (Dobson, 1979), and also in round-tailed ground squirrels (Dunford, 1977). Armitage and Downhower (1974) hypothesized that yearling yellow-bellied marmots disperse to avoid aggression from pregnant and lactating adult females. Holekamp (1984a), however, argued that aggression doesn't cause dispersal in males, though she suggested that aggression may facilitate dispersal. Neuhaus (2006) also suggested aggression from adults directed at yearling males may be related to dispersal in Columbian ground squirrels. In contrast, Festa-

Blanchet and King (1984) argued that adult aggression targeted at yearling males may be a way to reduce competition for resources and mates. Hypothetically, parents may benefit by forcing some of their offspring to disperse in order to reduce competition between siblings (Bélichon et al., 1996). A similar sentiment is echoed by Waser et al. (2013), who suggest that the only benefit of dispersal for juvenile banner-tailed kangaroo rats is to avoid competition with kin. In the study, banner-tailed kangaroo rat dispersers did not gain any measurable benefits, including better habitat, increased survival or life expectancy, increased offspring production and fecundity, or a reduction in inbreeding depression.

Consequences of dispersal and benefits of inbreeding

In some species, such as the banner-tailed kangaroo rat, dispersal does not seem to result in many benefits (Waser et al., 2013). Some proposed potential dispersal costs include risk of infection of unfamiliar pathogens and skills that are useful in the previous environment but not in the new environment, but these particular costs are mostly theoretical with little support (Pusey and Wolf, 1996). What's not theoretical, and has ample support, is the idea that the process of dispersal is particularly stressful and dangerous during the transience and settlement stages (Bélichon et al., 1996; Pusey and Wolf, 1996; Lawson Handley and Perrin, 2007). In Belding's ground squirrels, emigrants weigh less than non-dispersers during their juvenile summer (Holekamp, 1984b). Holekamp (1984b) believed this may be a result of the high energetic demands of emigration that occur when emigrants search for and store resources in an unfamiliar environment. Columbian ground squirrel males that remain residents emerge heavier and later than emigrants; this enables them to avoid aggression from territorial males during mating season, avoid exposure to late snow storms, and wait until better plants for foraging are available (Neuhaus, 2006).

Beyond health detriments of dispersal, dispersers and emigrants also face predation and the possibility of not finding a new population. When Columbian ground squirrels disperse, they follow trails and drainage systems to increase their chances of finding a new population (Wiggett and Boag, 1989). Despite this, one study estimated that survival rates are between 11% and 30% for female emigrants and between 16% and 26% for male emigrants (Wiggett and Boag, 1989). Juvenile banner-tailed kangaroo rats that remain philopatric are four times more likely to survive compared to individuals that are within population dispersers (Waser et al., 2013). Surplus arctic ground squirrels are driven from breeding territories between May and August and from pre-hibernation territories from August to November, at which point they are forced to enter refugee populations (Carl, 1971). During the forced breeding emigration, individuals are at high risk of predation from foxes, and during the forced pre-hibernation emigration, individuals are subject to bear predation.

Even if individuals do manage to live through the transience stage of dispersal and find a new population, they must then deal with unfamiliar territory and the potential of aggression from conspecifics. In the new territory, immigrant individuals are at a disadvantage with resource competition because they may be less dominant or may not know where to find the resources necessary for survival and fitness (Lawson Handley and Perrin, 2007). In regards to examples related to aggression, in round-tailed ground squirrels, aggression does not influence resident male dispersal, but it does prevent potential immigrants from settling in a population (Dunford, 1977). In fact Dunford (1977) saw so few immigrants during his study that he suggested that they may be chased out of the population so quickly that they’re never seen.

Similar results were found in one study on California ground squirrels, in which all chases observed from adult, female residents were directed towards young immigrants (Dobson, 1979).

Conversely, emigrating individuals may have still experienced aggression from conspecifics if they had remained residents. Support for this idea is mixed, with some research supporting fitness and safety benefits, rather than danger, for residents. In yellow-bellied marmots, social stability and production of young in a colony is maximized by resident males remaining in the population for an extended period of time (Armitage and Downhower, 1974). In Columbian ground squirrels, the presence of closely related kin in the population results in significant fitness benefits for females, possibly through lowered aggression and increased behavioral cooperation (Dobson et al., 2012). More specifically, agonistic interactions are lower among uterine kin than non-uterine kin, and agonistic interactions are lower in littermate sisters compared to non-littermate sisters (King, 1989). Females of the species tend to live in close proximity to mothers and close kin, and although females do not give their home range territory to their daughters, they may shift their territory or share close spaces (Arnaud et al., 2012). Arnaud et al. (2012) proposed that one reason fewer agonistic interactions occur between Columbian ground squirrel female kin is that they have a decreased need to be territorial. Females are less likely to kill their own mother or daughter’s offspring, and the decreased risk of infanticide leads to lowered territoriality and aggression and, in turn, lowered energy expenditure. In addition to reducing the chances of infanticide, Arnaud et al. (2012) suggested that living near close kin may decrease an adult’s own predation risk because individuals with close kin may be more likely to sound with alarm calls when potential threats are present. Through field observations Fairbanks and Dobson (2010) supported the opposite conclusion, finding that the presence of kin does not affect vigilance in Columbian ground squirrels. Despite this, females, such as Columbian ground squirrels, still may particularly benefit by remaining residents and by remaining near their kin (Lawson Handley and Perrin, 2007; Dobson et al., 2012). As indicated by these examples, entire populations may benefit by retaining some level of individuals as residents. Still, there are exceptions to this. In meerkats, although females are believed to be able to recognize relatives, they do not perform nepotistic behaviors like increased vigilance, baby-sitting, and pup feeding (Leclaire et al., 2012).

Although, some researchers argue that dispersal will lead to increased fitness, including comparatively more mates and stronger offspring (Lidicker, 1962), other research and modeling has suggested dispersers actually have decreased mating opportunities (Lemel, 1997). Even if individuals don’t disperse, attempts to avoid inbreeding, and pickiness about mating partners, may result in missed mating opportunities (Lehmann and Perrin, 2003). One study found that some black-tailed prairie dog females that refused to mate with closely related males in their coterie lost an entire breeding season when they were unable to access other males (Hoogland, 1992). In the same study, when moderately related black-tailed prairie dog individuals did mate, no inbreeding depression occurred. A moderate level of inbreeding is tolerated in polygynous species, like black-tailed prairie dogs and yellow-bellied marmots, as long as reproductive costs are low and individuals aren’t suffering from decreased reproductive success (Hoogland, 1992; Olsen et al., 2012).

A moderate level of inbreeding, or optimal inbreeding, may actually be genetically beneficial for populations and individuals (Pusey and Wolf, 1996; Puurtinen, 2011). Outbreeding depression is a decreased fitness in offspring that results from breeding between individuals from populations with genetic differentiation (Pusey and Wolf, 1996). Subspecies and populations that are spatially isolated may have different local adaptations or phenology, such as breeding timing; mating between individuals of different subspecies or isolated populations could break up gene complexes that are adapted to the local environment. While some examples of this occurring in plants and marine invertebrates exist, almost no examples of this phenomenon exist for vertebrates, including Columbian ground squirrels (Pusey and Wolf, 1996; Dobson and Murie, 1987). While researchers may believe outbreeding depression occurs in their study species, the differences in trait displays observed may just be phenotypic plasticity rather than locally adapted complexes; this idea is supported for Columbian ground squirrels (Dobson and Murie, 1987).

Goals, hypotheses, and predictions

The information above leads to an important question: is there a fitness difference between resident and immigrant males, in regards to number of mating partners and number of offspring produced? And if individuals of one residency status are fitter than the other, what traits and variables cause this difference? Columbian ground squirrels are a good model choice for this question for both practical and biological reasons. Practically, individuals of this species are easy to study because they are abundant, diurnal, easy to trap and mark, and live in easily accessible and visible locations, specifically plains and meadows (Holekamp, 1984a). As a result, we have a rich dataset of regularly and carefully monitored behavioral interactions, like mating pairs, and basic biological measurements, such as weights, phenological dates, and pedigrees.

Columbian ground squirrel females are philopatric, but males have mixed dispersal patterns (Murie and Harris, 1984). Some males emigrate to a new population as yearlings (or two-year-olds if lightweight), but others remain philopatric residents (Murie and Harris, 1984; Festa-Blanchet and King, 1984). Studies have reported different levels of male philopatry, with one study measuring 17% of males as residents (Murie and Harris, 1984), and in another, 70% of males were residents (Neuhaus, 2006). Due to the proportion of males remaining philopatric, the polygynandrous mating system, and the multiple paternity that occurs in some litters, this species has a high potential for inbreeding (Raveh et al., 2011; Murie, 1995).

The goal of this study was to determine if residency status affects fitness, specifically the number of mating partners and offspring produced. We additionally wanted to detect which traits and variables cause the difference in fitness, if a difference did exist. The findings of this study can then be used to explain possible reasons for the dispersal pattern of Columbian ground squirrels. Additionally, understanding the effects of residency status and dispersal patterns on fitness in this species may be beneficial to understanding the effects of dispersal on fitness in other mammalian species that are more difficult to study. To achieve this goal, we proposed the following hypotheses and predictions. Multiple variables could contribute to the number of mating partners and offspring produced, therefore multiple hypotheses may be simultaneously supported.

Hypothesis 1: Resident adult males weigh more, on average, because individuals in the population recognize them and because they're more familiar with their habitat (and better able to locate food). Because they're recognized, they experience less aggression and higher acceptance from females. Resident males then waste less energy on agonistic interactions and spend more time foraging, eating, gaining weight, and mating.

Prediction 1: Resident adult males will have a higher average weight and more mating partners than immigrant adult males. Weight will have a significantly positive relationship to number of mates and number of offspring produced.

Alternative Hypothesis 1: Immigrant adult males will have a higher average weight and more mating partners than resident adult males because the healthiest males are the only ones able to emigrate successfully. Immigrant males will be challenged less frequently by resident males and will be more dominant due to their heavier weight and will therefore spend less time on agonistic interactions and more time foraging, gaining weight, and mating.

Alternative Prediction 1: Immigrant adult males will have a higher average weight and more mating partners than immigrant adult males. Weight will have a significantly positive relationship to number of mates and number offspring.

Hypothesis 2: Resident adult males will have an earlier emergence date than immigrant adult males because younger immigrant males will emerge, or "appear," later to avoid aggression from resident males during mating season. As a result, many adult immigrant males, particularly younger ones, will miss mating opportunities, and resident males will have a higher number of average mating partners than immigrant males.

Prediction 2: Resident adult males will have an earlier average emergence date and more mating partners than immigrant adult males. Emergence date will have a significantly negative relationship to number of mating partners and offspring produced. Age will have a significantly positive relationship with emergence date.

Hypothesis 3: Resident adult males will be older, on average, than immigrant adult males because immigrant adult males will have a shorter life span due to the stresses of emigration. Older males get more mating partners, possibly because they are more dominant, therefore resident adult males will have a higher number of average mating partners than immigrant males.

Prediction 3: Resident adult males will have a higher average age and more mating partners than immigrant adult males. Age will be related to number of mating partners and number of offspring produced; as age increases, the number of mating partners and number of offspring produced significantly increases.

Alternative Hypothesis 3: There will be no difference in average age of resident adult males and immigrant adult males because they experience relatively similar environmental and social conditions throughout their lives. Age is related to number of mates because older males are more dominant and get more mates, but because there is no difference in average age, there will be no difference in average number of mating partners or offspring produced.

Alternative Prediction 3: Immigrant adult males and resident adult males will have a similar average age and have similar number of average mating partners and offspring produced. Age will be significantly positively related to number of mates.

Hypothesis 4: The number of years a male has been in the population affects the number of mating partners he has and offspring he produced. As a female becomes more familiar with a male, she will be more willing to mate with him. Resident adult males will have a higher average number of years in the population because immigrant adult males will live a shorter length of time in the population due to aggression directed at them in their first year and due to the stresses of emigration that shorten their lifespan.

Prediction 4: Resident adult males will have a higher average number of years in the population compared to immigrant adult males. During the first year as adults in the population, resident adult males will be significantly more likely to mate than immigrant first-year adult males. After the first year, there will be no difference in number of mating partners and offspring produced of resident adult males and immigrant adult males.

Methods

Data collection, study species, and study site

Columbian ground squirrels (*U. columbianus)* live in alpine and sub-alpine meadows in the Rocky Mountains in the north-western United States and south-western Canada (Elliot and Flinders, 1991). This ground-dwelling sciurid species is colonial, diurnal, active from approximately mid-April to early August, and hibernates the rest of the year (Elliot and Flinders, 1991; Michener, 1984; Dobson and Murie, 1987; Dobson et al., 1992). They are a polygynandrous species that exhibits multiple paternity, and they spend the first few weeks of the active season mating, during which each female undergoes estrous during five or six hours a single day (Raveh et al., 2011; Murie, 1995; Elliott and Flinders, 1991). We studied a population of Columbian ground squirrels in Sheep River Provincial Park, Alberta, Canada (110°W, 50°N; 1550 m elevation) from 1992 to 2015 during their active seasons. The population included both philopatric, resident males (i.e. males that were born in the population and remained as adults) and immigrant males (i.e. males that immigrated from different populations, most likely from a population in of the several nearby meadows). If a male emigrates to a new population and becomes an immigrant, he usually does so at the end of his yearling summer (Neuhaus, 2006). Reports of the percentage of Columbian ground squirrels that emigrate varies, with Neuhaus (2006) reporting that 65% of yearling males and 35% of yearling females "disappear" and Murie and Harris (1984) estimating that 83% of males and 13% of females emigrate.

We included data collected from 2005 to 2015 in this analysis because detailed mating logs were collected for ten of these eleven years and paternity for all resident individuals was known. This dataset included 56 adult males aged two years and older and 90 adult mating females. Some of these individuals lived in the population multiple years, resulting in a sample size of 127 male-years and 244 female-years (191 of which had mating logs). We measured the variance of number of mates and number of offspring for each individual male using intraclass correlation tests; these tests showed low repeatability (see results section) justifying analysis of data using samples rather than individuals. We refer to these samples as “male samples” and “female samples” for simplicity.

Each year, we recorded the following information for each male: age, emergence date and location, mating partners, and the number of offspring produced. For resident males, we determined age based on the year they were born in the population. We estimated the ages of immigrant male at first observation based on their weight and what time in the active season they entered the population; in general they were recorded as two years old unless they were particularly heavy (500 g or more), in which case they were sometimes recorded as three years old. We recorded emergence date, location, and number of mating partners through visual observations. In the analysis, we measured emergence as the number of days an individual emerged after the emergence date of the first squirrel of that year. Paternity of each pup born in the population was determined through DNA testing, which allowed us to measure the number of offspring produced by each male each year. For DNA analysis, 13 microsatellite loci were amplified using polymerase chain reaction (PCR) for each individual, and CERVUS 3.0 (Marshall et al., 1998; Kalinowski et al., 2007) was used to assign paternity. Tissue for the analysis came from a short tail biopsy of approximately 1 mm, which was preserved in 95% EtOH until analysis was performed (further analysis details described in Raveh et al., 2010).

Data analyses

We performed all analyses in R statistical software version 3.0.1 (R Core Team, 2013). For these analyses, close female relatives included only littermate sisters and mothers. Columbian ground squirrel littermate siblings are only 39% genetically related due to multiple paternity, in which each litter may contain both full and half siblings (Raveh et al., 2010). Other female relatives, therefore, such as aunts, cousins, and daughters, have the potential to be equally related, or more so, than mothers and littermate siblings. However, sciurid species, including Columbian ground squirrel males, are most likely to be capable of recognizing uterine kin, possibly by using odor cues (Steiner, 1974; Davis, 1984; Raynaud and Dobson, 2011). Due to this, of the females that males interact with, they are most likely to recognize and respond to the presence of their mother and littermate sisters, rather than aunts, daughters, and female cousins.

We tested the predictions related to mass and emergence of males using identical methods. Two separate Welch two sample t-tests compared the average mass between immigrant male samples (n=56) and resident male samples (n=71), and we performed the same tests for average emergence. Binomial models examined the effect of mass and emergence on mating likelihood in separate tests; these analyses included only male samples with confirmed mating status (i.e. it was known whether each mated with one or more females; n=124 male samples). Two separate linear models calculated the effect of mass on number of mating partners (n=113 male samples) and the number of offspring produced (n=127 male samples), and we measured possible interactions between mass and residency status. We created these same models using emergence in place of mass. Due to the distribution of residuals for the linear regressions of the effects of emergence on number of mating partners and on number of offspring produced, we also performed these analyses with polynomial regression models. Additionally, for these polynomial regression models, we tested for interactions between residency status and emergence and between residence status and the polynomial term. We also measured the effect of age on emergence date through a polynomic regression model to better assess the hypothesis and prediction regarding the effects of emergence on mating partners (n=127 male samples); we also tested for interactions between residency status and age in regards to emergence.

To test predictions related to age, we performed a Welch two sample t-test comparing the average ages of adult resident male samples (n=71) and immigrant males samples (n=56). Prior to analysis, we detected a dramatic difference in mating opportunities and offspring produced between two-year-old males and three-year-old males, therefore we first analyzed average number of mating partners and average number of offspring produced between two-year-old male samples (n=38 male samples for mating test and n=44 male samples for offspring test) and three-year-old males (n=31 male samples for mating test and n=34 male samples for offspring test) in two Welch two sample t-tests. Similar to mass and emergence, we used general linear models to measure the effect of age (only including males aged three years and older) on number of mating partners (n=75 male samples) and number of offspring produced (n=83 male samples) and tested for possible interactions of age and residency status.

Because length of time in the population was hypothesized to influence mating opportunities, we performed two Pearson's chi-squared tests (with Yates' continuity correction) to determine whether two-year-old immigrant male samples (n=18) or first-year immigrant male samples (n=23) were more similar to two-year-old resident male samples (n=23) based on mating probability. To further support the results of the chi-squared tests (which indicated what group of immigrant males was most similar to two-year-old resident males), a follow up Welch two sample t-test compared the average number of mating partners of two-year-old resident samples (n=8) and first-year immigrant samples (n=6), including only individuals that mated with one or more females.

Due to the results of that t-test, we compared the average number of years an individual had been in the population, as an adult, that year between adult resident male samples (n=71) and immigrant male samples (n=56) in a Welch two sample t-test. (In regards to how we determined the number of years an individual had been in the population, both two-year-old resident male samples and first-year immigrant male samples were recorded as one year in the population as adults, for example, while three-year-old resident male samples and second-year immigrant male samples were listed as two years in the population as adults, etc.) Because of the differences in mating habits of first-year adults and second-year adults, we performed two Welch two sample t-tests: one compared the average number of mating partners of first-year male samples (n=43) to second-year male samples (n=27) and the other compared the average number of offspring of those two groups (n=49 and n=31, respectively). For males that had been in the population for two years or more, we measured the relationship between length in population and number of mating partners (n=70 male samples) and the relationship between length in population and number of offspring produced (n=78 male samples) through two separate linear models, and we additionally tested for interactions between length of time in the population and residency status.

To test the predictions regarding differences in mating partners and offspring produced between resident and immigrant males, we performed two Pearson's chi-squared tests (with Yates' continuity correction). One of these tests compared the observed and expected counts of males mating with more or more females between immigrant male samples (n=54) and adult resident male samples (aged two years and older, n=70), and the other compared the observed and expected proportion of males that produced one or more offspring between immigrant (n=56) and adult resident male samples (n=71). We compared the average number of mating partners of all resident adult male samples (aged two years and older, n=63 male samples) and all immigrant male samples (n=50 male samples) and compared the average number of mating partners of only resident male samples and immigrant male samples that were confirmed as mating with one or more females (n=51 male samples and n=31 male samples, respectively) using two Welch two sample t-tests. We performed two more Welch two sample t-tests: one compared the average number of offspring produced between all resident, adult male samples (aged two years and older, n=71 males) and all immigrant male samples (n=56 males). The other compared the average number of offspring produced by only immigrant male samples and resident male samples that were confirmed mating with one or more females (n=35 male samples and n=58 male samples, respectively). To further understand the association between mating, offspring, and residency status, we created two linear models. One measured the relationship between number of mates and resulting offspring produced of all adult male samples, including those that didn't mate (n=113 male samples), and the other determined the same relationship with only male samples that mated with one or more females were included (n=82 males samples). Interactions between residency status and number of mating partners and number of offspring were additionally calculated for both.

Results

Repeatability: mates and offspring

We measured the variance of the number of mating partners within each individual male (n=54 individual males; n=113 male samples) between years using a random effects model, and the results indicated low repeatability. We observed that for number of mating partners, the repeatability was 0.079 (among group variance =2.166 [±2.42; ±95% CI], total variance = 27.411). We also measured the variance in number of offspring produced within each individual male (n=56 individual males, n=127 males samples) through a random effects model. Similar to number of mates, for number of offspring produced, the repeatability was also low at 0.093 (among group variance= 3.762 [±6.11; ±95% CI], total variance = 40.283). For both variables, because the confidence interval for the among group variance overlapped zero, the repeatability was judged to be extremely low, and the assumption of statistical independence among years for individual males was supported.

Mass

A Welch two sample t-test indicated there was no significant difference in average mass at emergence from hibernation in the spring between immigrant male samples (496.8±82.8 SD g) and adult resident male samples (504.9±81.7 SD g) (d.f.=117.47, 95% CI[-21.00, 37.29], p=0.581; Figure 2.1). Through a generalized linear model, we found that for each 1 g increase in mass, male samples were 1.02 times more likely to mate (1.01, 1.03; 95% CL) with one or more females (n=124 males, $p<0.001$). Through a linear model, we found that for each 1 g increase in mass, male samples had a 0.03 mate (±0.01, ±95% CI) increase in number of mating partners ($r^2=0.30$, d.f.=111, $p<0.001$; Figure 2.2); there was no interaction between residency status and mass in regards to number of mates (d.f.=109, p=0.138). Similarly, we found that for each 1 g increase in mass, male samples had a 0.03 offspring (±0.01; ±95% CI) increase in number of offspring produced ($r^2=0.15$, d.f.=125, $p<0.001$; Figure 2.3), and there was no interaction between residency status and mass in regards to number of offspring produced (d.f.=123, p=0.833).

Emergence

Resident adult male samples emerged from hibernation in the spring significantly earlier than immigrant male samples (or, in the case of new immigrant male samples, were initially seen in the population, rather than emerged) by an average of 4.9 days (6.4±7.9 SD days compared to 11.2±10.1 SD days, respectively; d.f.=101.90, 95% CI [-8.13, -1.60], p=0.004; Figure 2.4). Through a generalized linear model, we found that for each 1 day later in emergence, a male samples was 0.82 times as likely to mate (0.77, 0.89; 95% CL) with one or more females (n=124, $p<0.001$). A general linear model showed that for each 1 day later in emergence, immigrant male samples had a 0.3 mate (±0.11; ±95% CI) decrease in the number of total mates that year ($r^2=0.35$, d.f.=48, $p<0.001$), while resident male samples had a 0.5 mate (±0.11; ±95% CI) decrease in the number of total mates that year ($r^2=0.52$, d.f.=61, $p<0.001$; Figure 2.5a). We observed a significant interaction between emergence and residency status, such that the slope of the emergence/number of mates relationship was 0.16 mates per 1 day later smaller in immigrant male samples relative to resident male samples (d.f.=109, p=0.048). Due to the uneven distribution of residuals of the data when a linear model was created, we also analyzed the data as a polynomic regression; however, both models had similar goodness of fits, as indicated by the r-squared values. The polynomic regression model of the effect of emergence on number of mating partners showed that for adult resident male samples, for every one day later in emergence, there was a 0.8 mate (±0.25; ±95% CI) decrease in number of mating partners (r^2=0.51, d.f.=108, p<0.001, Figure 2.5b) with a significant polynomic curve of a 0.01 mate [±0.01; ±95% CI] increase in mating partners beginning around emergence day 25 (p=0.002). We found observed no interaction between neither residency status and emergence date (d.f.=108, p=0.738), nor residency status and polynomic curve (d.f.=108, p=0.802).

In regards to offspring produced, we found that for every 1 day later in emergence, male samples had a 0.4 offspring (±0.10; ±95% CI) decrease in offspring produced (r^2=0.26, d.f.=125, p<0.001; Figure 2.6a). We observed no interaction between residency status and emergence date in regards to number of offspring produced (d.f.=123, p=0.795). Due to the residuals of the data, we also performed a polynomic regression, and the goodness of fit was slightly better, with a higher r-squared value for the polynomial model. The polynomic regression model of the effect of emergence on number of offspring showed that for adult male samples, for every one day later in emergence, there was a 0.8 offspring (±0.34; ±95% CI) decrease in number of offspring produced (r^2=0.31, d.f.=122, p<0.001, Figure 2.6b) with a significant polynomic curve of a 0.01 offspring (±0.01; ±95% CI) increase in offspring beginning around emergence day 25 (p=0.014). We observed no interaction between neither residency status and emergence date (d.f.=122, p=0.408), nor residency status and polynomic curve (d.f.=122, p=0.436).

When the effects of age and age-squared on emergence, and an interaction term between age and residency, were included in a regression model, results showed that for every one year increase in age, there was an 11.7 day (±3.77; ±95% CI) decrease in emergence date (r^2=0.34, d.f.=123, p<0.001; Figure 2.24) with a significant polynomic curve of a 1.1 day (±0.42; ±95% CI) increase in emergence date (i.e. later emergence) beginning around five-years-old for immigrant samples and six-years-old for resident samples. We observed an interaction between residency status and age, such that the slope of the age/emergence relationship was 1.2 days smaller resident adult male samples relative to immigrant adult male samples (d.f.=123, p=0.004). There was no interaction between residency status and the age polynomic curve when it additionally was included in the model (d.f.=122, p=0.300). Due to the significant interactions between residency and age, we also performed separate polynomic regression models for residents and immigrants. We found that for every one-year increase in age in adult resident male samples, there was a 10.8 day (±4.0; ±95% CI) decrease in emergence date (r^2=0.34, d.f.=68. P<0.001; Figure2.24) with a significant polynomic curve of a 0.9 day (±0.42, ±95% CI) increase beginning around age 5 (d.f.=68, p<0.001). For adult immigrant male samples, for every one year increase in age, there was a 15.8 day (±11.95; ±95% CI) decrease in emergence date (r^2=0.27, d.f.=53, p=0.011; Figure 2.24) with a nearly significant polynomic curve of a 1.6 day (±1.61; ±95% CI) increase beginning around age 5 (d.f.=68, p=0.055).

Age

We found a near statistically significant difference in average age between adult resident male samples (3.7±1.8 years) and adult immigrant male samples (3.2±1.2 years) (d.f.=121.58, 95% CI [-1.03, 0.03], p=0.06; Figure 2.7). Of the 41 two-year-old male samples, 31.7% mated, compared to 96.4% of male samples aged three years or older (n=83 males). Of the two-year-old samples, 11.1% of the immigrants mated (n=18 males), whereas 47.8% of the residents mated (n=23 males). Including all two and three-year-old male samples, a Welch two sample t-test indicated there was a significant difference in number of mates of two-year-old male samples and three-year-old male samples (0.7±1.6 SD mates compared to 8.1±4.5 SD mates, respectively; d.f.=36.47, 95% CI [-9.04, -5.62], p<0.001; Figure 2.8). For male samples aged three years and older, for every 1-year increase in age, male samples had a 0.1 mate (±0.65; ±95% CI) increase in number of mates that year, but that difference was not statistically significant (r^2=-0.01, d.f.=73, p=0.670; Figure 2.9). We found no interactions between residency status and age for male samples aged three years and older in regards to number of mates (d.f.=71, p=0.71).

Results for the relationship between age and offspring produced were similar to that of age and number of mates. A Welch two sample t-test found a significant difference in the number of offspring produced between two-year-old male samples (0.6±1.3 SD offspring) and three-year-old male samples (7.8±7.0 SD offspring) (d.f.=34.73, 95% CI [0.59, 7.82], p<0.003; Figure 2.10). A general linear model showed that, for male samples aged three years and older, for every 1 year increase in age, male samples had a 0.4 offspring (±0.98; ±95% CI) decrease in the number of offspring produced that year, but that difference was not statistically significant (r^2=-0.005, d.f.=81, p=0.436; Figure 2.11). We observed no interactions between residency status and age for male samples aged three years and older in regards to number of offspring produced (d.f.=79, p=0.368).

Length of time in the population

A Pearson's chi-squared test (with Yates' continuity correction) comparing mating of resident two-year-old male samples and immigrant two-year-old male samples indicated significant differences between observed and expected values, such that two-year-old resident samples were 4.31 times more likely to mate compared to two-year-old immigrant samples (47.8% mating compared to 11.1% mating, respectively; Pearson's χ^2=4.705, d.f.=1, n=41, p=0.030; Table 2.1). Residency status for two-year-old male samples and whether a male mated had a moderate association (φ=0.339). When the same test was used to compare the mating status of two-year-old resident male samples and first-year immigrant male samples, however, although two-year-old resident samples were 1.83 times more likely to mate than first-year immigrant samples, there was no significant difference between observed and expected values of whether a male sample mated (47.8% mating compared to 26.1%, respectively; Pearson's χ^2=1.493, d.f.=1, n=46, p=0.222; Table 2.2). A Welch two-sample t-test also showed that the average number of mates of two-year-old resident samples that mated (2.8±2.1 SD) was similar to that of first-year immigrant samples that mated (4.7±4.1 SD) (d.f.=6.98, CI [-2.45, 6.28], p=0.334; Figure 2.12). Thus, first-year immigrant samples, rather than immigrant samples estimated to be two years old, are most similar to two-year-old resident male samples in their mating habits.

Through a Welch two-sample t-test, we found a significant difference between the average number of years an individual sample had been in the population, as an adult, between adult resident male samples (2.7±1.8 SD years) and adult immigrant male samples (2.0±1.1 SD years) (d.f.=119.94, CI [-1.20, -0.16], p=0.011; Figure 2.13). Of the male samples that were first-year adults, 37% mated (n=46), compared to 97% of male samples that were in the population as second-year adults or older (n=78). Related to those percentages, a Welch two-sample t-test showed that male samples that were in their first year in the population as an adult had, on average, 7.5 fewer mates compared to male samples in their second year as adults (1.2±2.4 SD mates compared to 8.6±4.1 SD mates, respectively; d.f.=37.65, CI [-9.24, -5.70], p<0.001; Figure 2.14). For male samples that had been in the population for two years or more, a general linear model (including an interaction for years in population and residency) indicated that for every 1 year increase in the number of years in the population, there was a 0.04 mate (±0.64; ±95% CI) increase in the total number of mates that year, but the change was not statistically significant (r^2=-0.01, d.f.=68, p=0.913; Figure 2.15). We observed no interactions for residency status and number of years in the population in regards to number of mates (d.f.=66, p=0.966).

Similar to number of mates, a Welch two-sample t-test showed that male samples in their first year in the population as an adult produced an average of 7.5 fewer offspring when compared to male samples in their second year (0.9±1.7 SD offspring compared to 8.4±7.0 SD offspring, respectively; d.f.=32.33, CI [-10.16, -4.92], p<0.001; Figure 2.16). We found, through a general linear model, that for male samples, excluding first-year male samples, for every 1 year increase in the number of years in the population, there was a 0.5 offspring (±1.01; ±95% CI) decrease in the total number of offspring produced that year, however the decrease was not statistically significant (r^2=-0.001, d.f.=76, p=0.335; Figure 2.17). We observed no interaction between residency status and years in the population in regards to offspring produced (d.f.=74, p=0.438).

Mates and offspring produced

Through a Pearson's chi-squared test (with Yates' continuity correction), we found a significant difference between the observed and expected counts of individuals that mated based on residency status, such that adult resident male samples were 1.28 times more likely to mate compared to immigrant male samples (64.8% of immigrants mating with one or more female compared to 82.9% of residents; Pearson's χ^2=4.374, d.f.=1, n=124, p=0.036; Table 2.3). Although residency status and mating status were dependent variables, the association was weak (φ=0.188). When all male samples, regardless of mating status, were included in a Welch two sample t-test, adult resident male samples had 2.6 more mates, on average, when compared to immigrant male samples (7.4±5.1 SD mates compared to 4.7±5.1 SD mates, respectively; d.f.=105.28, CI [-4.54, -0.71], p=0.008; Figure 2.18). When only individuals that mated were included, however, there was no significant difference in number of mates between adult resident male samples (9.1 ±4.0 SD mates) and immigrants male samples (7.6±4.4 SD mates) (d.f.=58.93, CI [-3.39, 0.49], p=0.14; Figure 2.19).

Using a Pearson's chi-squared test (with Yates' continuity correction), we found a significant difference between the observed and expected counts of individuals producing offspring based on residency status, such that adult resident male samples were 1.33 times more likely to produce offspring compared to immigrant male samples (76.1% of residents producing one or more offspring compared to 57.1% of immigrants; Pearson's χ^2=4.294, d.f.=1, n=127, p=0.038; Table 2.4). Similar to residency and mating status, the relationship between residency status and whether a male sample produced offspring was also weak (φ=0.184). Despite an increased proportion of resident samples producing offspring, per results of a Welch two sample t-test, there was no significant difference in the average number of offspring produced between adult resident male samples (6.1±6.0 SD offspring) and immigrant male samples (5.0±6.8 SD offspring) (d.f.=111.05, CI [-3.37, 1.20], p=0.348; Figure 2.20). The same non-significant difference of average number of offspring produced is found when the test is performed with only male samples that mated (8.0±7.1 SD offspring for immigrant male samples and 7.4±5.9 SD offspring for resident male samples; d.f.=61.79, CI [-2.30, 3.38], p=0.705; Figure 2.21).

Through a general linear model, when all male samples with known number of mates were included, we found that for every 1 mate increase in number of mates of a male sample, there was a 1.0 offspring (±0.15; ±95% CI) increase in number of offspring produced that year by that male sample (r^2=0.58, d.f.=111, p<0.001; Figure 2.22). Further modeling found a trend, but no significant interaction between residency status and number of mates in regards to number of offspring produced (d.f.=109, p=0.092). Due to the trend, the effects of number of mating partners of offspring produced were analyzed separately by residency. We found that for adult resident male samples, for every one mate increase in number of mating partners, there was a 0.9 offspring (±0.22; ±95% CI) increase in number of offspring produced (r^2=0.50, d.f.=61, p<0.001). For adult immigrant male samples, for every one mate increase in number of mating partners, there was a 1.1 offspring (±0.22; ±95% CI) increase in number of offspring produced (r^2=0.69, d.f.=48, p<0.001). Similar results were found when only male samples with one or more mates were included in the model: for every 1 mate increase in the number of mates, there was a 1.0 offspring (±0.26; ±95% CI) increase in the number of offspring produced by the male that year (r^2=0.42, d.f.=80, p<0.001; Figure 2.23). Further modeling indicated there was no significant interaction between residency status and number of mates in regards to offspring produced (d.f.=78, p=0.183).

Discussion

We found that adult, resident males were significantly more likely (1.28 times) to mate and 1.33 times more likely to produce offspring compared to adult, immigrant males. When all adult males were included in the analysis, resident males had significantly more mates than immigrant males (7.4 mates on average compared to 4.7), but there was no difference in number of offspring produced. When males that did not mate were excluded from the analysis, there was still no difference in offspring produced, and although the difference was no longer statistically significant, residents still had a trend towards more mating partners.

Mating partners and offspring produced were found to have a linear relationship; for every one mate increase in mating partners, there was a one offspring increase in offspring produced. This fits with the finding that adult, resident males were both more likely to mate and more likely to produce offspring. However, we would also expect that because residents have significantly more mates, they would also have significantly more offspring. This is not accurate based on our results. We suggest that an explanation for this discrepancy may be inbreeding depression.

Only one instance of inbreeding with close female relatives (mothers and littermate sisters) was observed over a 10-year period of time in Columbian ground squirrels (Klase and Dobson manuscript, 2016). Related species, however, such as black-tailed prairie dogs, meerkats, and yellow-bellied marmots, do not avoid moderate inbreeding, such as mating with cousins (Olsen et al., 2012; Hoogland, 1992; Leclaire et al., 2012). Similar to Columbian ground squirrels, black-tailed prairie dogs avoid inbreeding with close kin, such as parents, offspring, and siblings, but several instances of black-tailed prairie dogs males mating with daughters have been observed (Hoogland, 1992). In our study, we did not account for inbreeding between cousins, fathers and daughters, or aunts and nephews. Columbian ground squirrels would be unlikely to recognize these female relatives because experiments and field observations suggest they can only recognize uterine kin and their mother (Steiner, 1974; Raynaud and Dobson, 2011). Perhaps Columbian ground squirrel males are mating with cousins, aunts, and daughters, which would result in at least a moderate level of inbreeding in the population, including amongst resident males.

Olsen et al. (2012) found that in yellow-bellied marmots, inbred offspring have a decreased survival rate. Several meta-analyses have supported this same finding in a variety of species and also have supported that inbreeding can cause sperm deformities and sterility (Pusey and Wolf, 1996; Allendorf and Leary, 1986). The discrepancy between number of mating partners and number of offspring produced in adult, resident males in Columbian ground squirrels may be related to moderate inbreeding and the fitness consequences, such as decreased offspring survival, sterility, and sperm deformities. When the interaction between residency status on the number of mates/offspring slope was analyzed, there was a non-significant trend in which immigrant adult males produced 0.2 more offspring per one mating partner increase relative to resident adult males. Future research could address whether moderate inbreeding occurs to further explain the interaction between residency and the mates/offspring produced relationship.

Despite the trend towards decreased offspring production, based on the results, adult, resident males do not experience decreased fitness (measured by number of mates and number of offspring) when compared to immigrant males. Not only do resident adult males have, on average, equivalent offspring and mating partners, but they actually have a higher probability of mating and producing offspring, and they have significantly more mating partners when all adult males are analyzed together. By remaining residents, Columbian ground squirrel males have equivalent overall fitness as immigrant males, and they also have higher chances of survival because the avoid they dangers of emigration (Waser et al., 2013; Pusey and Wolf, 1996; Bélichon et al., 1996), such as predation (Carl, 1971) not finding a new population (Wiggett and Boag, 1989), and aggression that prevents entrance to the population (Neuhaus, 2006; Dobson, 1979, Dunford, 1977).

In this study, we proposed several hypotheses and analyzed several variables that may help explain why resident adult males have more mating partners compared to immigrant adult males, particularly when only mating males are included. One set of alternative hypotheses predicted that either resident or immigrant adult males would weigh more, on average, and that weight would have a significant relationship with number of mating partners. Although mass was positively related to mates, there was no significant difference in mass of resident and immigrant adult males. Mass, therefore, does not account for the significant difference in mating partners, and hypotheses 1 and alternative 1 were not supported by the results.

The number of mating partners of a male was predicted to be affected by the number of years in the population because we hypothesized that females would be more willing to mate with males with which they're familiar. We predicted that first-year adult resident males would be more likely to mate and would have more mating partners than first-year immigrants because females are more familiar with the residents. Some species of female ground squirrels are particularly aggressive towards immigrant males (Dunford, 1977; Dobson, 1979). We found, however, that immigrant and resident first-year adult males were equally likely to mate, and they had similar numbers of mating partners. We hypothesized that after the first year, number of years in the population would not influence number of mates because females would be equally familiar with males of both residency statuses. Similar to what we predicted, 97% of males that had been in the population over one year as adults mated, while only 37% of first-year males mated. Additionally, after the first year in the population, there was no relationship between years in population and number of mating partners. Although these results support prediction 4, they do not provide support that female familiarity influences male mating opportunities. The results also don't explain why resident adult males had a higher number of mating partners. Therefore, hypothesis 4 was not supported.

Age, however, may provide a partial explanation for why resident males are more likely to mate and have more mating partners than immigrant males. After age three, there was no relationship between age and number of mating partners. However, for two-year-olds, resident males were seven times more likely to mate than immigrant males, with 47.8% of resident two-year-olds and only 11.1% of immigrant two-year-olds mating (11 of 23 resident two-year-olds mating and 2 of 18 immigrant two-year-olds mating). This is a contrast to studies that have assumed that two-year-olds mating in Columbian ground squirrels is rare, particularly given our results on residents (Neuhaus, 2006; Murie and Harris, 1984). Additionally, resident adult males were found to be nearly significantly older, on average, than immigrant adult males (3.7 years compared to 3.2 years). More years in the population after age two leads to significantly more mates, therefore resident adult males have an advantage regarding mating likelihood and number of mates. These results provide some support for prediction 3: resident males do live longer than immigrant males, but age is only related to number of mates between ages two and three.

Emergence date, measured as number of days emerged after the first male of the year, may also be related to the difference in mates between resident and immigrant adult males. On average, resident adult males emerged significantly earlier than immigrant male (4.9 days compared to 11.2 days). Emergence was found to be significantly negatively related to number of mates in adult males, in which every day later an individual emerged, he had 0.8 fewer mating partners. Age and emergence date also had a negative polynomic relationship, as well as an interaction with residency status such that each year increase in age resulted in a 10.8 day earlier emergence date for adult resident males and a 15.8 day earlier emergence for adult immigrant males. This steeper slope in the age/emergence relationship for adult immigrant males does not appear to ultimately compensate in terms of average emergence date because resident males still emerge earlier on average. This may be because immigrants are nearly significantly younger, on average, than residents. The average younger age of immigrant males indicates that there are a higher proportion of younger immigrants compared to resident males, thus the difference in slope has minimal impact on emergence date. This may be related to hypothesis 4 in which we thought that young immigrant males emerge later to avoid aggression from older males during the mating season (Neuhaus, 2006).

The results regarding average emergence date and average age of immigrant and resident males and the relationship between age and emergence date may explain why resident adult males get more mating partners than immigrant adult males. A higher proportion of two-year-old residents mate, and this may contribute to why resident adult males have a higher probability of mating. Resident adult males are nearly significantly older, on average, when compared to adult immigrant males. Age influences emergence date, and because residents are older on average, they're emerging earlier on average. Emergence date is significantly related to number of mates, thus resident adult males have a higher number of average mating partners, due to the effects of age and emergence, when compared to immigrant adult males. These results best support hypothesis 2, in which younger adult immigrant males emerge later to avoid aggression from adult males and lose mating opportunities as a result. The results also support some of the predictions derived from hypothesis 3, which stated that adult immigrants are younger on average due to shorter life spans from the stresses of emigration, and because older males get more mates, immigrant adults have fewer mating partners. These results can be contrasted to other similar studies on dispersal, emigration, and fitness.

Our findings about fitness of immigrants and residents are compatible with a meta-analysis on dispersal that included 38 species, primarily birds and mammals; Bélichon et al. (1996) found that after post-dispersal settlement, a relatively equivalent number of studies found that reproduction in dispersers was higher for residents compared to those that found it was lower for residents. Neuhaus (2006) found in his study on Columbian ground squirrels that females were equally likely to choose an adult resident as an adult immigrant for their first mating partner. Neuhaus' (2006) study, however, only accounted for first mating partners, not all mating partners, which could explain our dissimilar conclusions regarding resident males gaining a mating advantage. Waser et al. (2013) found that banner-tailed kangaroo rat dispersers have similar number of offspring as non-dispersers, which we also observed in Columbian ground squirrels. Another study with comparable findings is that on North American red squirrels, which found that dispersal after weaning caused no differences in future offspring production when compared to non-dispersers (Berteaux and Boutin, 2000). Overall, we found that, in Columbian ground squirrel males, there are no annual fitness consequences (measured through mating likelihood, number of mating partners, and number of offspring produced) to remaining a philopatric resident, and if, anything, residents may be fitter than immigrants, with a slight advantage in terms of mating likelihood and mating partners. The results of our study, like those described above, provide a better understand of how emigration in mammals affects fitness.

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Tables and Figures

Table 2.1. Pearson's chi-squared with Yates' continuity correction comparing observed and expected instances of two-year-old males mating with one or more females based on residency status, including observed counts of individuals that mated and did not mate, expected counts in parentheses, and standard residuals (Pearson's χ^2=4.705, d.f.=1, n=41, p=0.030).

	Two-year-old residents	Two-year-old immigrants	Total Observed
Mated			
Obs/Exp	11 (7.3)	2 (5.7)	13
Residual	1.37	-1.55	
Did not mate			
Obs/Exp	12 (15.7)	16 (12.3)	28
Residual	-0.94	1.06	
Total Observed	23	18	41

Table 2.2. Pearson's chi-squared with Yates' continuity correction comparing observed and expected instances of two-year-old resident males and first-year immigrant males mating with one or more females, including observed counts of individuals that mated and did not mate, expected counts in parentheses, and standard residuals (Pearson's χ^2=1.493, d.f.=1, n=46, p=0.222).

	Two-year-old residents	First-year immigrants	Total Observed
Mated			
Obs/Exp	11 (8.5)	6 (8.5)	17
Residual	0.86	-0.86	
Did not mate			
Obs/Exp	12 (14.5)	17 (14.5)	29
Residual	-0.66	0.66	
Total Observed	23	23	46

Table 2.3. Pearson's chi-squared with Yates' continuity correction comparing observed and expected instances of adult males (aged two years and older) mating with one or more females in a single year based on residency status, including observed counts of individuals that mated and did not mate, expected counts in parentheses, and standard residuals (Pearson's χ^2=4.374, d.f.=1, n=124, p=0.036).

		Immigrants	Residents	Total Observed
Mated				
	Obs/Exp	35 (40.5)	58 (52.5)	93
	Residual	-0.86	0.76	
Did not mate				
	Obs/Exp	19 (13.5)	12 (17.5)	31
	Residual	1.50	-1.31	
Total Observed		54	70	124

Table 2.4. Pearson's chi-squared with Yates' continuity correction comparing observed and expected instances of adult males (aged two years and older) producing one or more offspring in a single year based on residency status, including observed counts of individuals that produced offspring and did not produce offspring, expected counts in parentheses, and standard residuals (Pearson's χ^2=4.294, d.f.=1, n=127, p=0.038).

		Immigrants	Residents	Total Observed
Produced offspring				
	Obs/Exp	32 (37.9)	54 (48.1)	86
	Residual	-0.96	0.85	
Did not produce offspring				
	Obs/Exp	24 (18.1)	17 (22.9)	41
	Residual	1.39	-1.24	
Total Observed		56	71	127

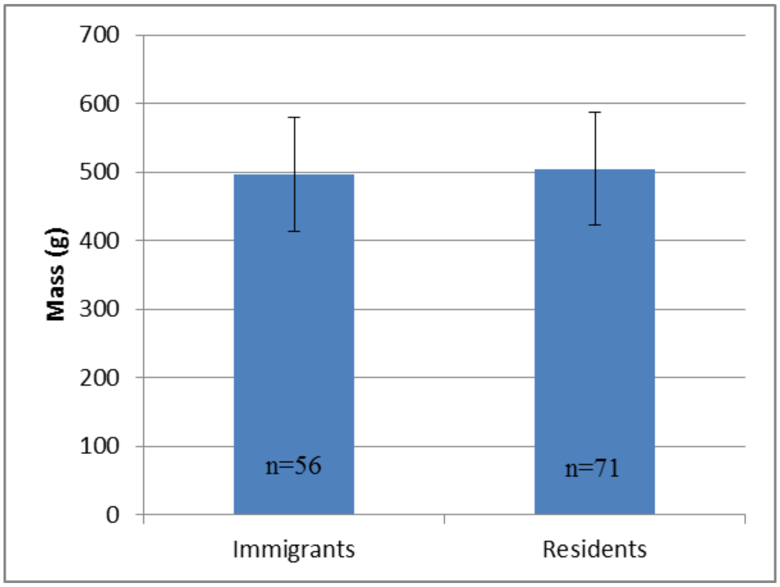

Figure 2.1. Average mass of adult males (aged two years and older), with standard deviation, based on residency status (Welch two sample t-test: d.f.=117.47, 95% CI [-21.00, 37.29], p=0.581).

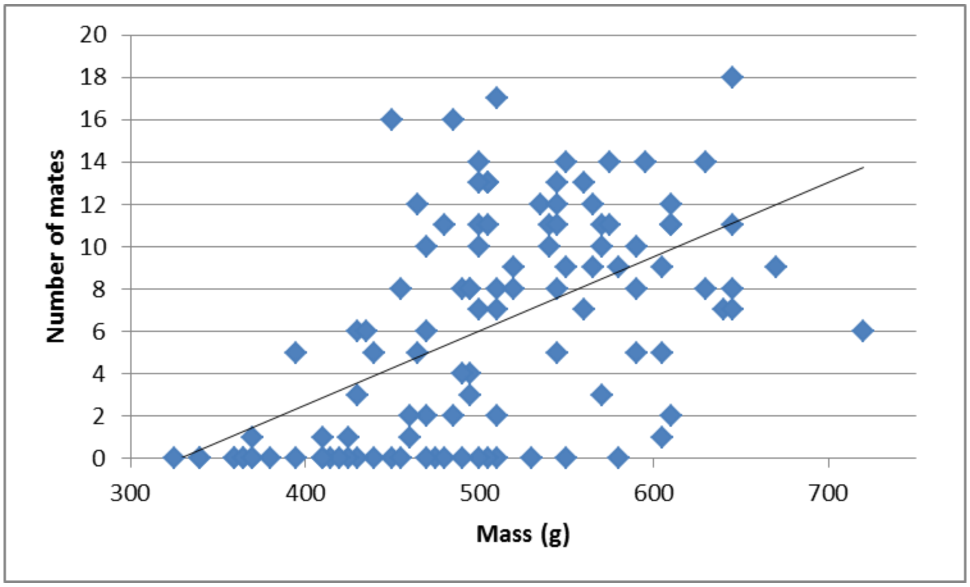

Figure 2.2. Relationship between mass and number of mating partners for adult males aged two years and older (r^2=0.30, d.f.=111, p<0.001).

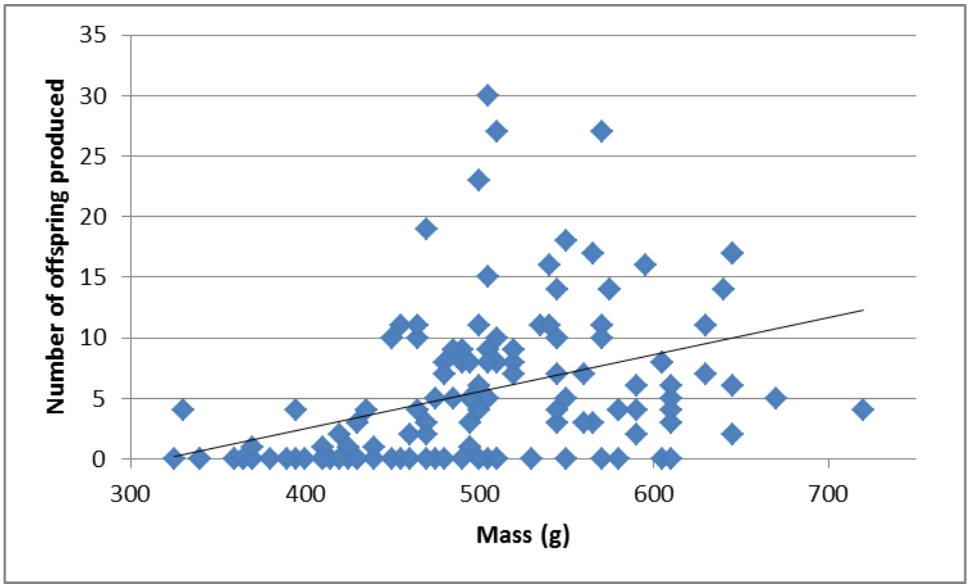

Figure 2.3. Relationship between mass and number of offspring produced for adult males aged two years and older (r^2=0.15, d.f.=125, p<0.001).

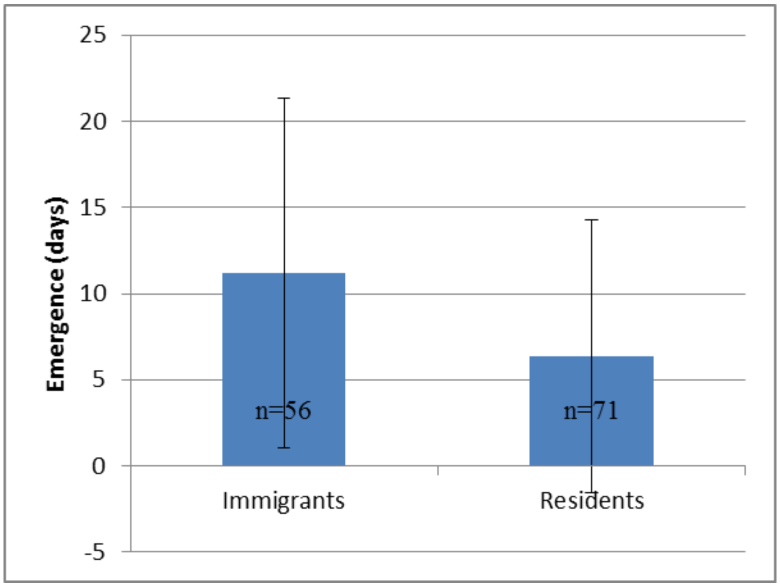

Figure 2.4. Average number of days emerged past the emergence date of the first squirrel that year of adult males aged two years and older, with standard deviation, based on residency status (Welch two sample t-test: d.f.=101.90, 95% CI [1.60, 8.13], p=0.004).

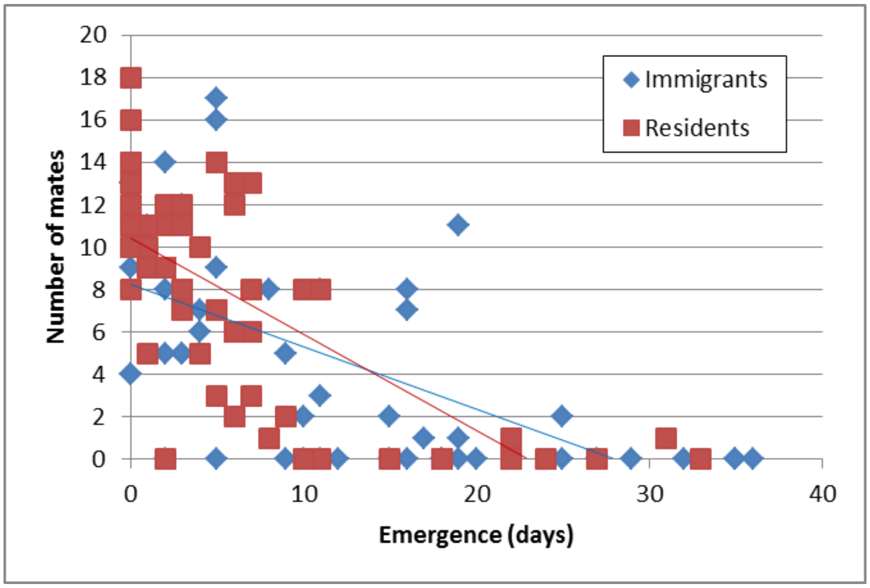

Figure 2.5a. Relationship (as a linear regression) between the number of days emerged past the emergence date of the first squirrel that year and the number of mating partners for adults two years and older (immigrants: r^2=0.35, d.f.=48, p<0.001; residents: r^2=0.52, d.f.=61, p<0.001).

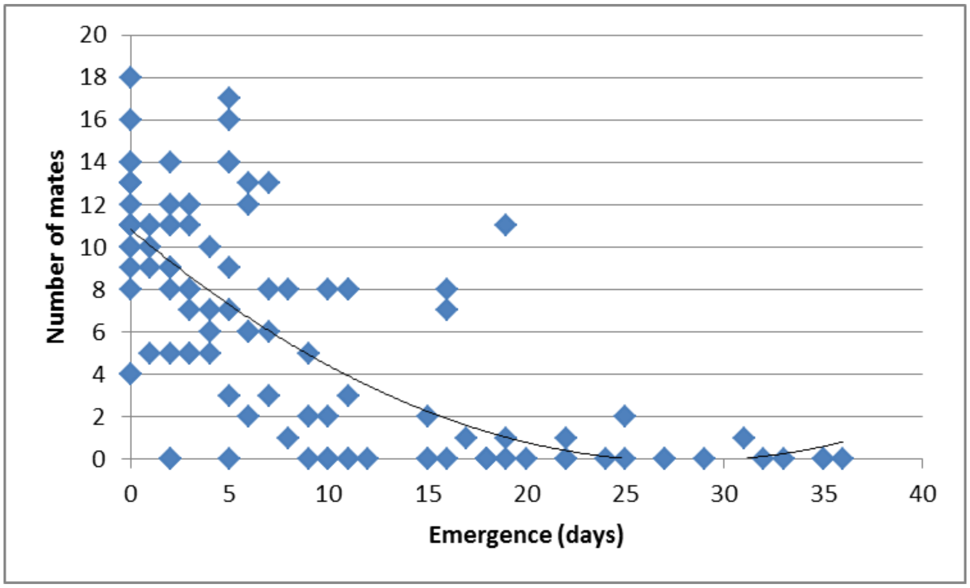

Figure 2.5b. Relationship (as a polynomial regression) between the number of days emerged past the emergence date of the first squirrel that year and the number of mating partners for adults two years and older (r^2=0.51, d.f.=108, p<0.001).

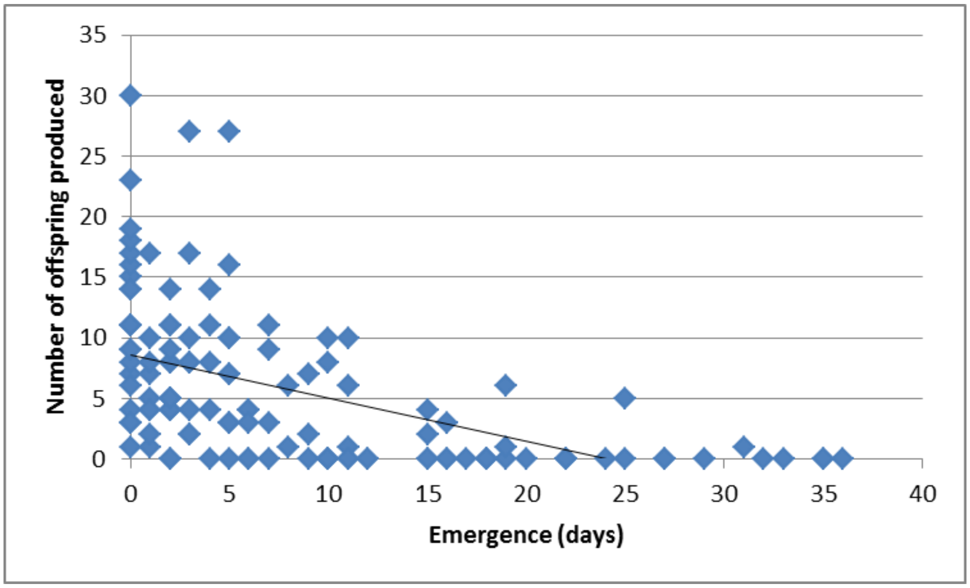

Figure 2.6a. Relationship (as a linear regression) between the number of days emerged past the emergence date of the first squirrel that year and the number of offspring produced for adults two years and older (r^2=0.26, d.f.=125, p<0.001).

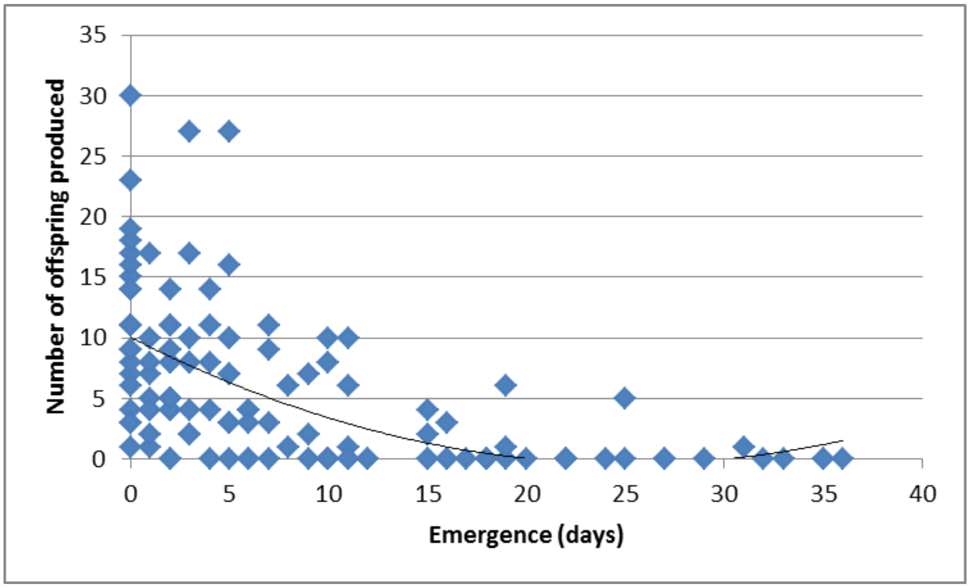

Figure 2.6b. Relationship (as a polynomial regression) between the number of days emerged past the emergence date of the first squirrel that year and the number of offspring produced for adults two years and older (r^2=0.31, d.f.=122, p<0.001).

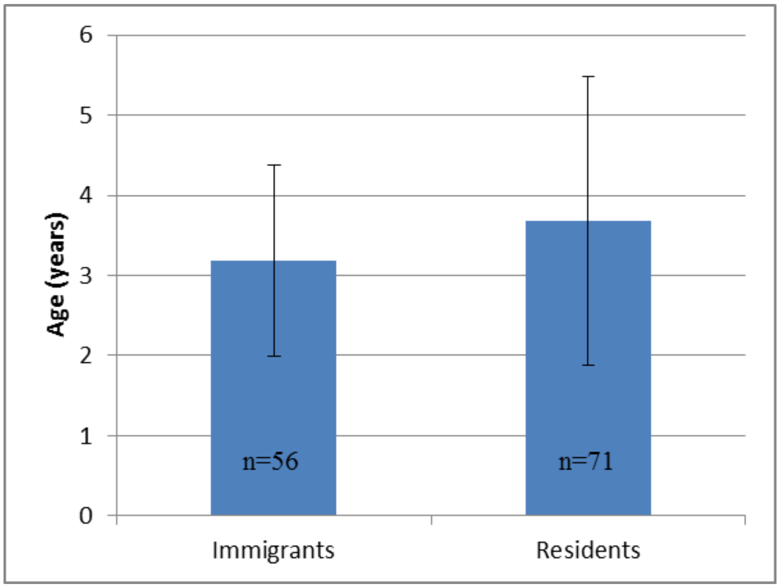

Figure 2.7. Average age of adult males aged two years and older, with standard deviation, based on residency status (Welch two sample t-test: d.f.=121.58, 95% CI [-1.03, 0.03], p=0.06).

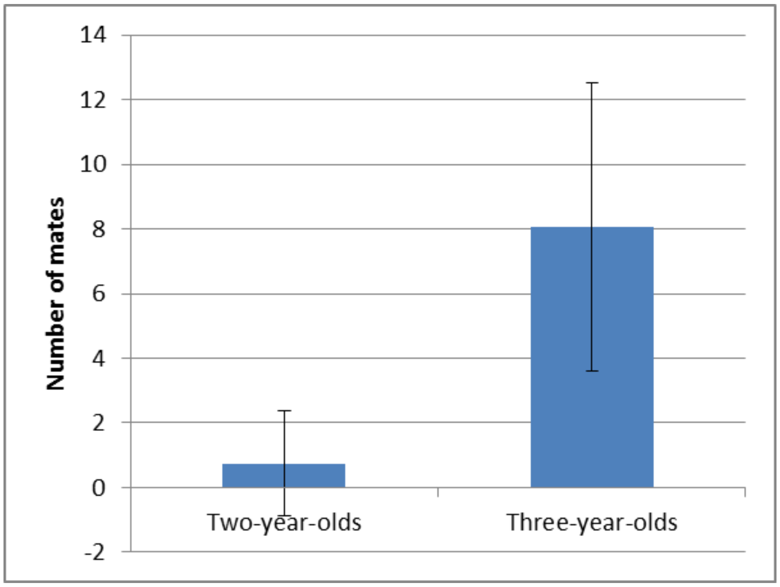

Figure 2.8. Average number of mating partners of two-year-old males and three-year-old males, with standard deviation (n=38 for two-year-olds and n=31 for three-year-olds; Welch two sample t-test: d.f.=36.47, 95% CI [-9.04, -5.62], p<0.001).

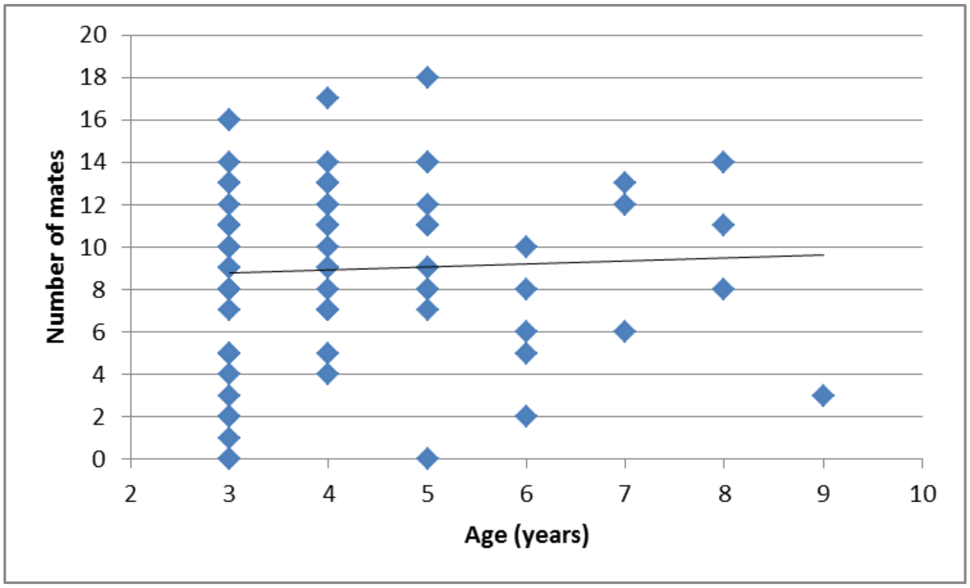

Figure 2.9. Relationship between age and number of mating partners for males aged three years and older (r^2=-0.01, d.f.=73, p=0.670).

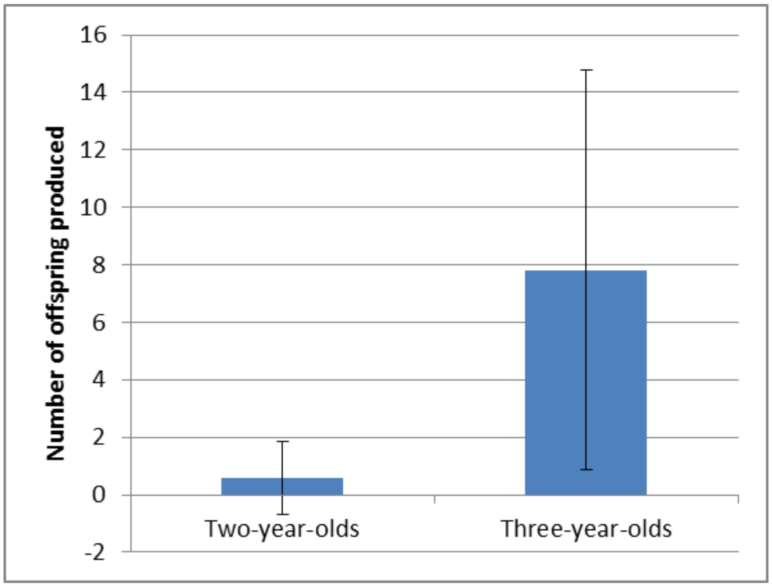

Figure 2.10. Average number of offspring produced by two-year-old males and three-year-old males, with standard deviation (n=44 for two-year-olds and n=34 for three-year-olds; Welch two sample t-test: d.f.=34.73, 95% CI [0.59, 7.82], p<0.003).

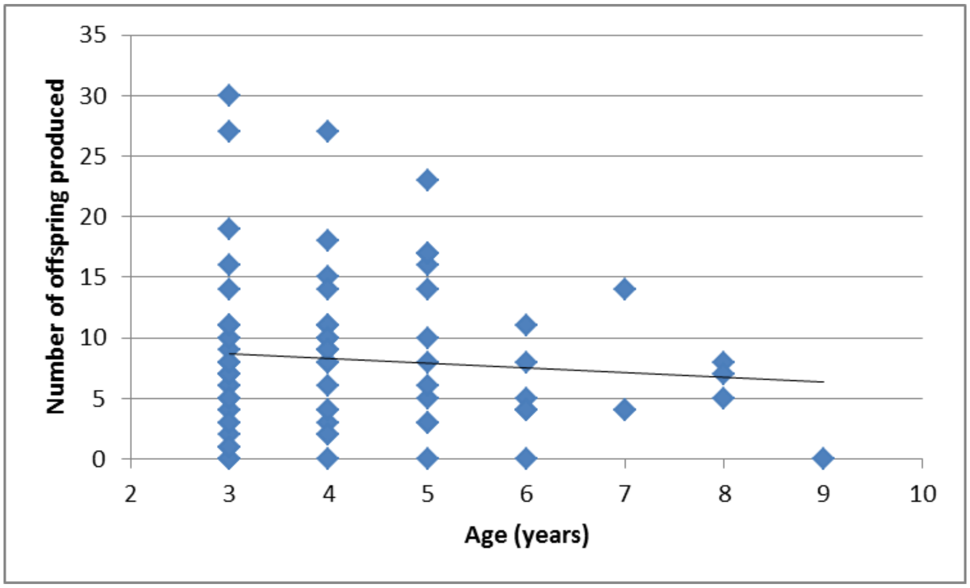

Figure 2.11. Relationship between age and number of offspring produced that year by males aged three years and older (r^2=-0.005, d.f.=81, p=0.436).

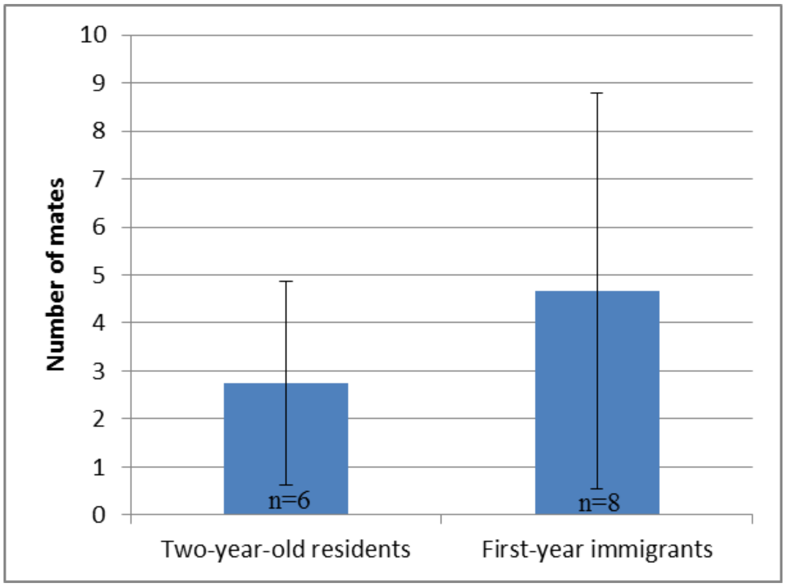

Figure 2.12. Average number of mating partners of two-year-old resident males and first-year immigrant males, with standard deviation (Welch two sample t-test: d.f.=6.98, CI [-2.45, 6.28], p=0.334).

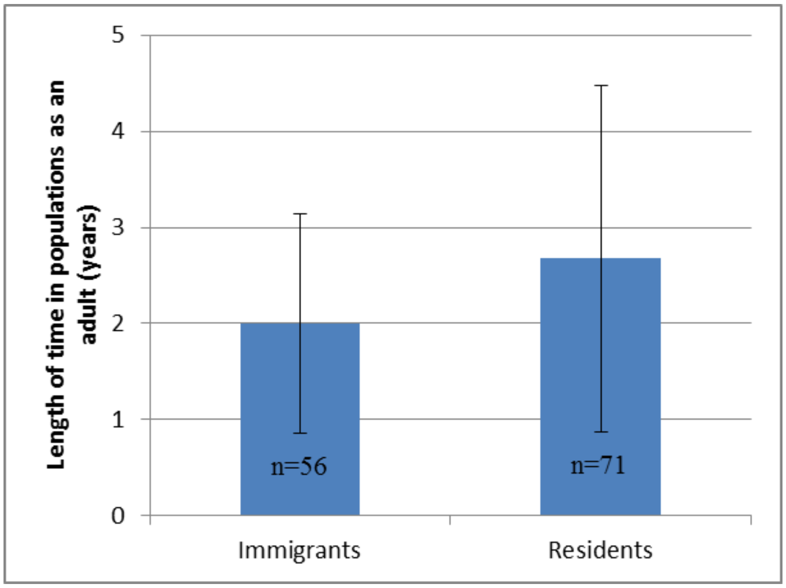

Figure 2.13. Average number of years males aged two years or older had been in the population as adults, based on residency status, with standard deviation (Welch two sample t-test: d.f.=119.94, CI [-1.20, -0.16], p=0.011).

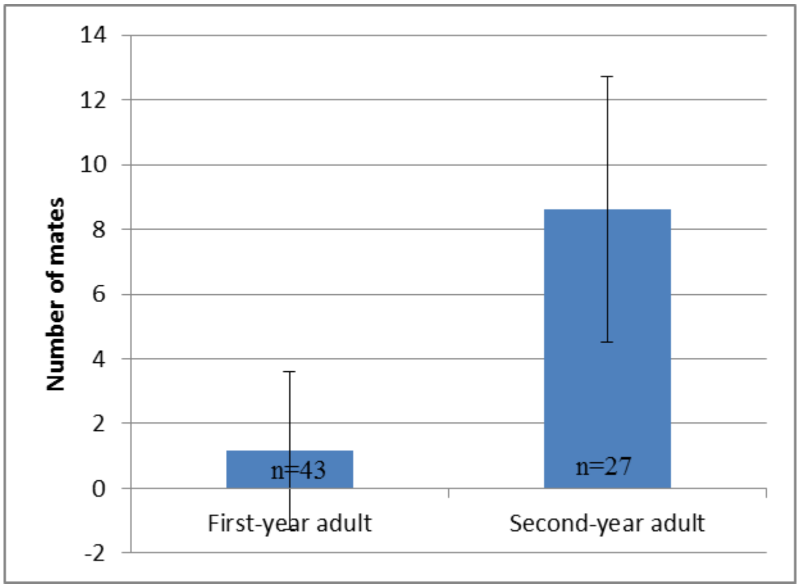

Figure 2.14. Average number of mating partners of males that had been in the population as adults for their first year compared to their second year, with standard deviation (Welch two sample t-test: d.f.=37.65, CI [-9.24, -5.70], p<0.001).

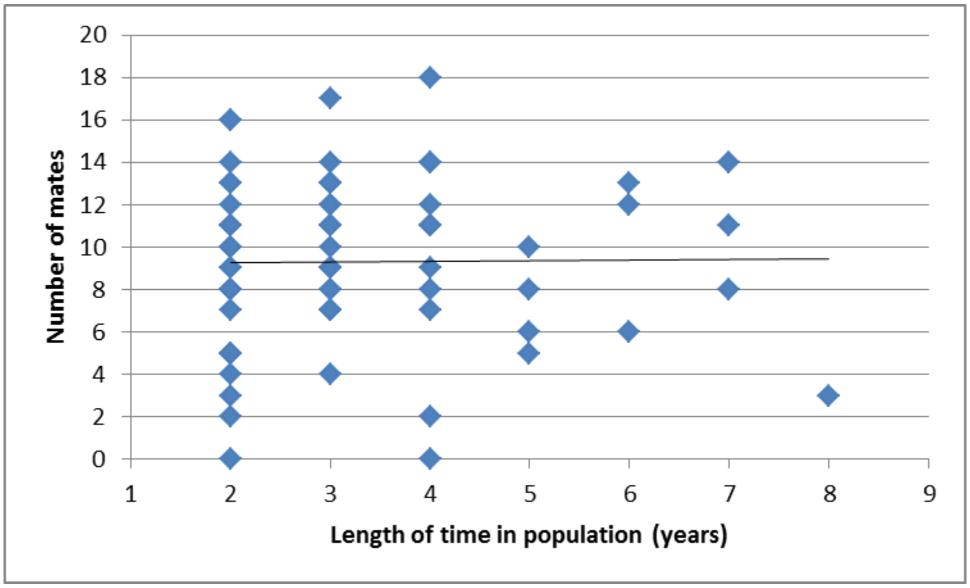

Figure 2.15. Relationship between the number of years a male had been in the population compared to the number of mating partners he had that year, including adult males that had been in the population two years and longer (r^2=-0.01, d.f.=68, p=0.913).

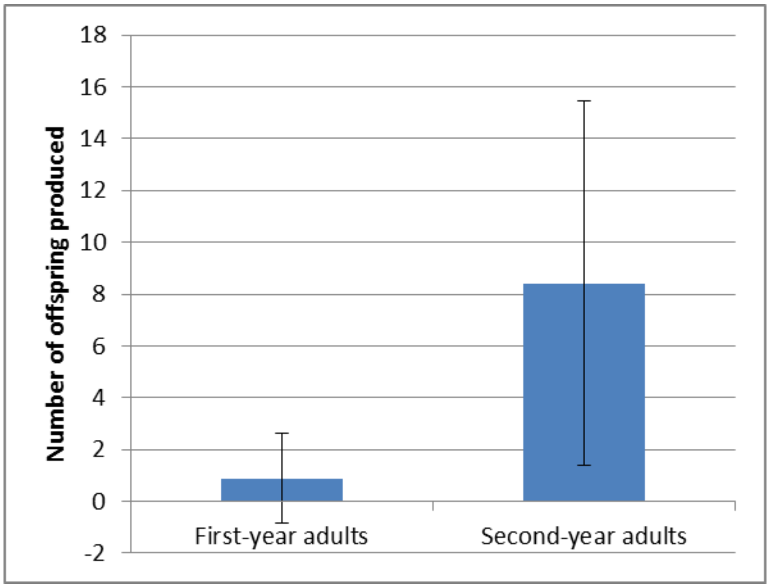

Figure 2.16. Average number of offspring produced by males that were in the population as an adult for the first year compared to two years as an adult, with standard deviation (Welch two sample t-test: d.f.=32.33, CI[-10.16, -4.92], p<0.001; n=49 for first-year adults and n=31 for second-year adults).

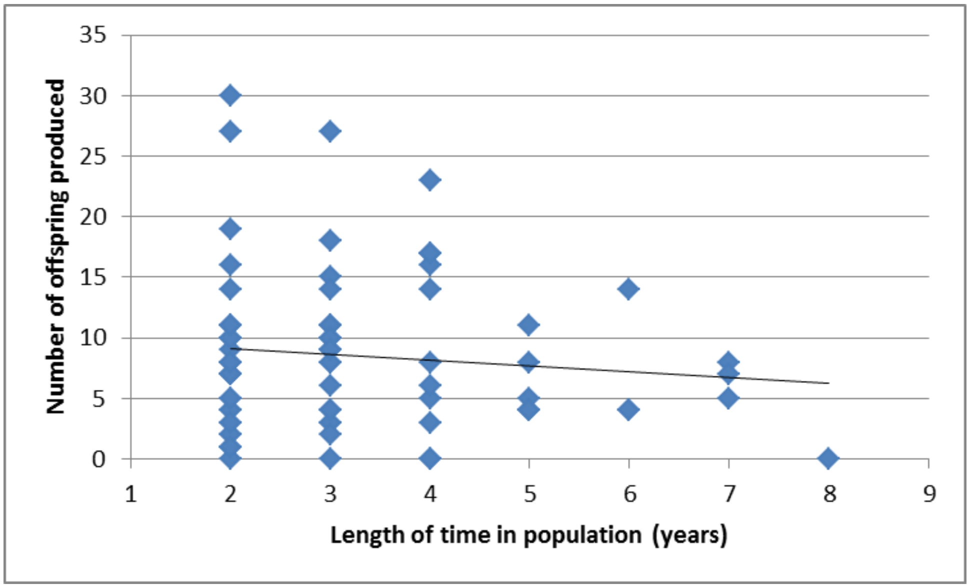

Figure 2.17. Relationship between the length of time a male had been in the population as an adult and the number of offspring he produced that year (r^2=-0.001, d.f.=76, p=0.335).

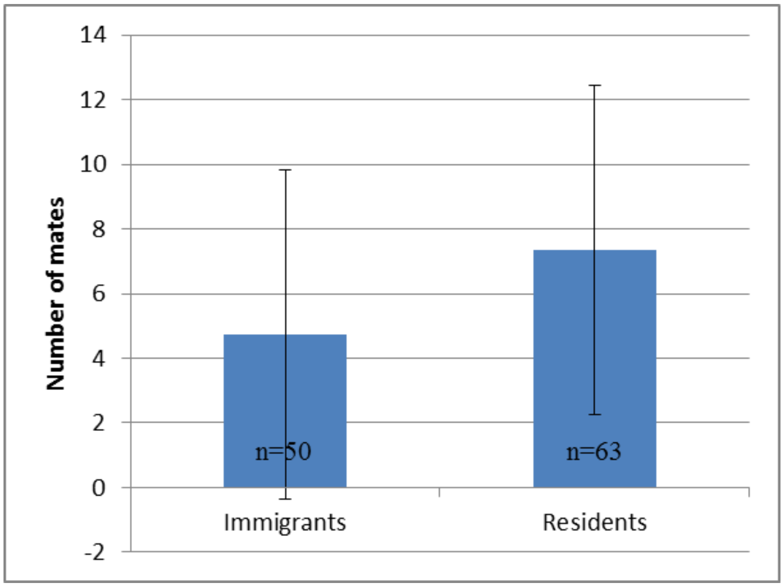

Figure 2.18. Average number of mates in a year of all individual adult males, aged two years and older, based on residency status, with standard deviation (Welch two sample t-test: d.f.=105.28, CI [-4.54, -0.71], p=0.008).

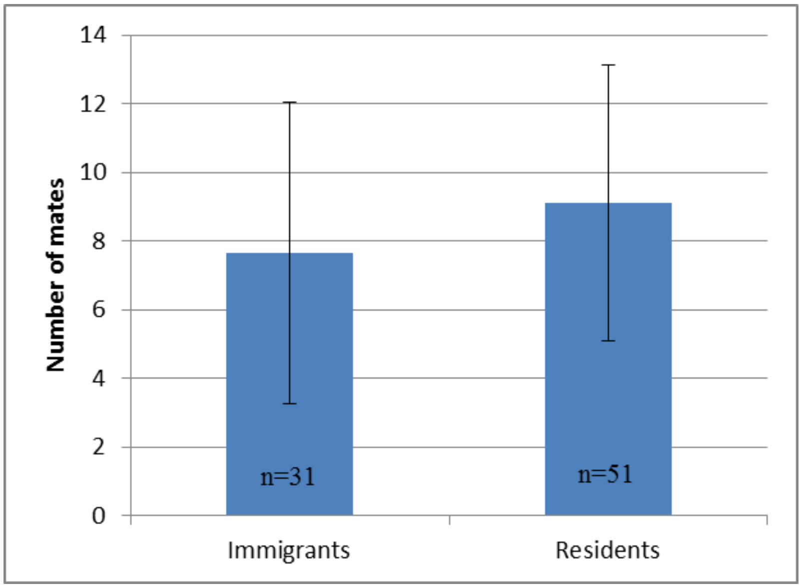

Figure 2.19. Average number of mates in a year of adult males, aged two years and older, that mated that year, based on residency status, with standard deviation (Welch two sample t-test: d.f.=58.93, CI [-3.39, 0.49], p=0.14).

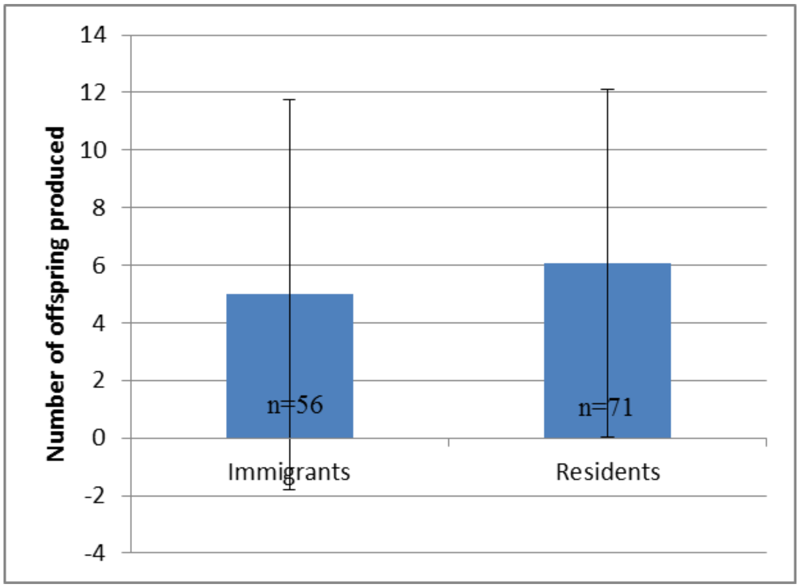

Figure 2.20. Average number of offspring produced in a year by all individual adult males, aged two years and older, based on residency status, with standard deviation (Welch two sample t-test: d.f.=111.05, CI [-3.37, 1.20], p=0.348).

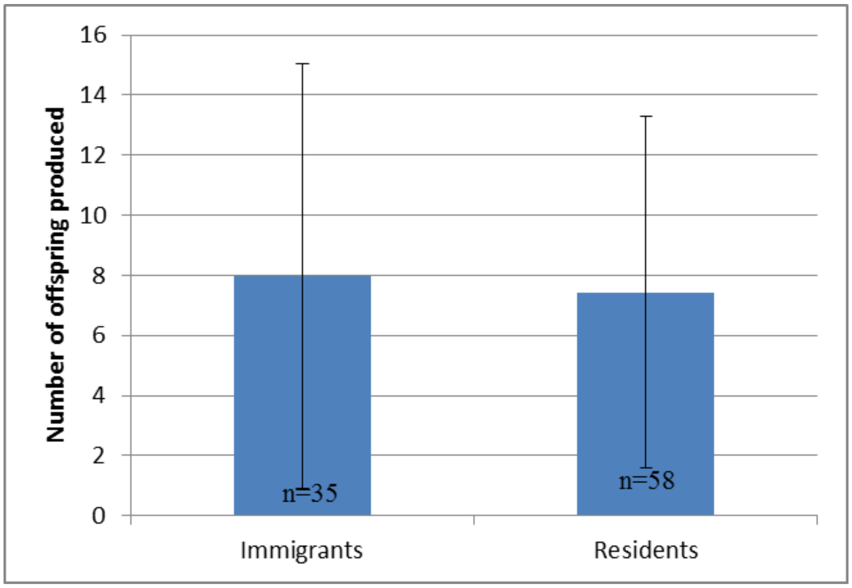

Figure 2.21. Average number of offspring produced in a year by adult males, aged two years and older, that mated that year, based on residency status, with standard deviation (Welch two sample t-test: d.f.=61.79, CI [-2.30, 3.38], p=0.705).

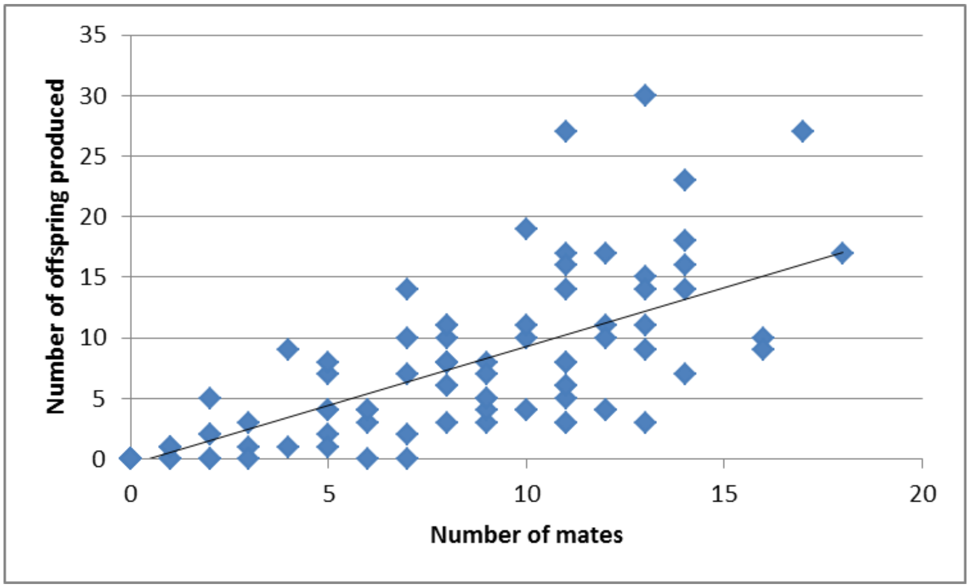

Figure 2.22. Relationship between number of mating partners in a year, including all males regardless of mating status, and number of offspring produced that year (r^2=0.58, d.f.=111, p<0.001).

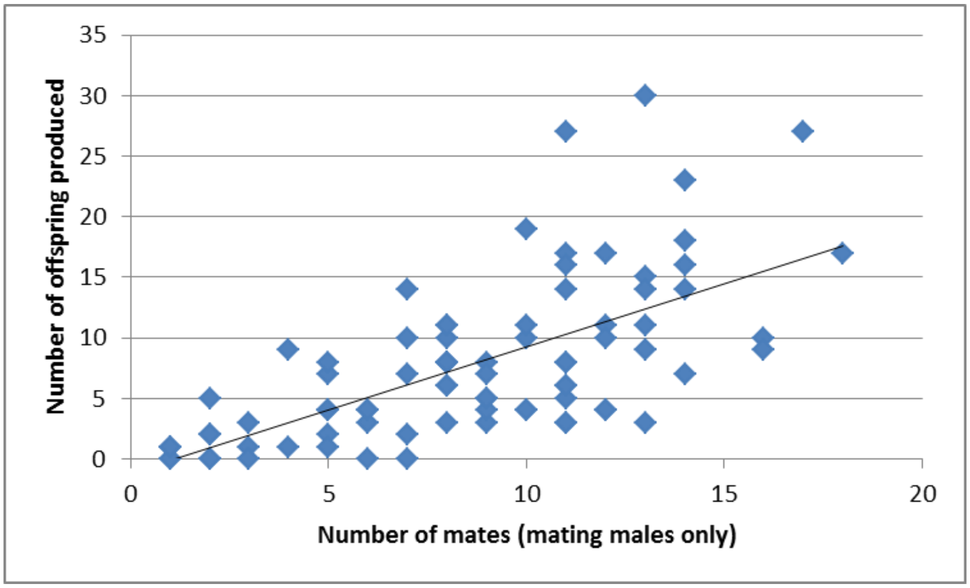

Figure 2.23. Relationship between the number of mating partners that year, including only males that mated that year, and number of offspring produced (r^2=0.42, d.f.=80, p<0.001).

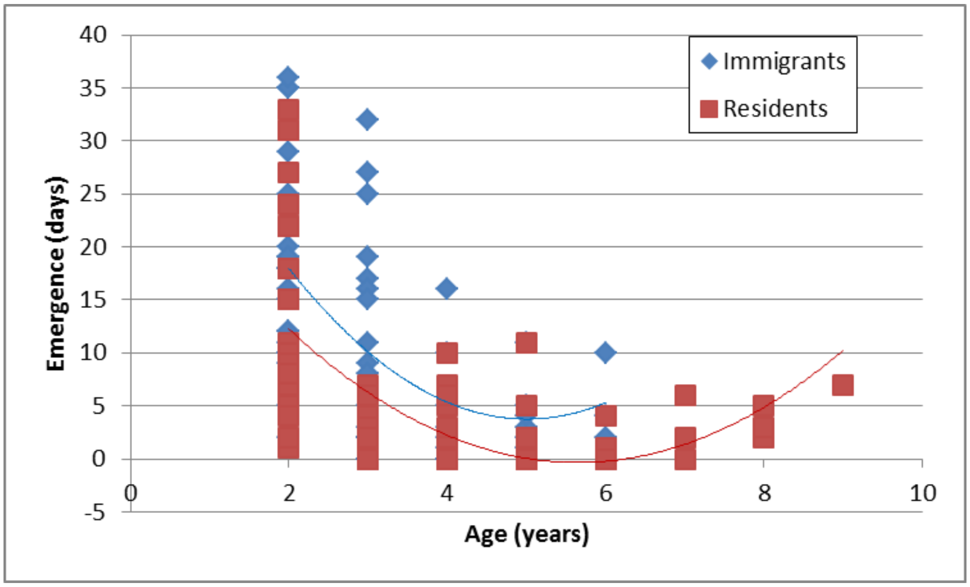

Figure 2.24. Relationship between age and the number of days emerged past the emergence date of the first squirrel that year for adults aged two years and older (immigrants: r^2=0.27, d.f.=53, p=0.011; residents: r^2=0.34, d.f.=68, p<0.001).