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Sociality, Bateman's gradients, and the polygynandrous genetic mating system of round-tailed ground squirrels (*Xerospermophilus tereticaudus*)

Karen E. Munroe · John L. Koprowski

Abstract Historically, most mammals have been classified as polygynous; although recent molecular evidence suggests that many mammals may be polygynandrous, particularly the ground-dwelling sciurids. We genotyped 351 round-tailed ground squirrels (*Xerospermophilus tereticaudus*) using seven microsatellite loci to determine paternity in 31 litters from 2004 to 2007. Polygyny was evident in all years except in 2007, when the population size was reduced. Multiple paternity occurred in the majority of litters (55%) with 2.5 ± 0.26 sires/litter ($n=31$). Forty-nine percent of resident males ($n=114$) sired offspring, and of males that sired offspring ($n=56$) 27% sired young in multiple litters in a single breeding season. Litter size was positively correlated with the number of sires. Through an indirect analysis of paternity, we found 21 litters (68%) with an average relatedness of 0.5 or less. Males had a greater opportunity for sexual selection ($I_s=1.60$) than females ($I_s=0.40$); Bateman's gradient was also greater in males (1.07 ± 0.04, $n=56$) than females (0.82 ± 0.08, $n=31$). The mating system in round-tailed ground squirrels defined through genetic analyses and Bateman's gradients is polygynandrous compared to the previously suggested polygynous mating system as established by behavioral observations and fits within the predictions of the ground squirrel sociality models. Upon evaluating the predictions of the sociality models among sciurid species, we found a negative relationship between the level of sociality with litter size and the average percentage of multiple paternity within a litter. Thus, recent genetic information and reclassification of mating systems support the predictions of the ground-dwelling squirrel sociality models.

Keywords Arizona · Microsatellites · Multiple paternity · Parentage analysis · Polygynandry

Introduction

Mating systems are defined by patterns of reproductive behaviors that are influenced by spatial and temporal distributions of reproductively receptive females. Female aggregation affects the reproductive strategies of males, specifically their ability to acquire and monopolize mates (Bradbury and Vehrencamp 1977, Emlen and Oring 1977, Clutton-Brock 1989). In polygynous mating systems, ecological factors create aggregations of reproductive females where males are likely to succeed in monopolizing mating opportunities (Emlen and Oring 1977). However, where males are unable to monopolize mating opportunities, mating systems instead tend to be polygynandrous, where neither sex is restricted to a single mate within a breeding season (Emlen and Oring 1977). The potential fitness benefits to females mating with multiple males in polygynandrous systems include fertility assurance by reducing genetic incompatibility (Zeh and Zeh 1996, 1997, 2001; Jennions and Petrie 2000), increasing genetic diversity of offspring (Loman et al. 1988, Ridley 1993, Jennions and Petrie 2000), and avoiding aggressive behaviors from courting males (Jennions and Petrie 2000, Wolff and Macdonald 2004). Furthermore, post-copulatory sexual selection may occur through sperm competition

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(Parker 1970) or cryptic female choice (Møller and Birkhead 1989, Birkhead 2000).

Traditional observational studies of mating systems often focus on male behaviors (Clutton-Brock 1989, Shuster and Wade 2003), particularly the ability of males to monopolize access to reproductive females or resources required for mating such as food or breeding sites (Emlen and Oring 1977). Mating systems were classically defined by the number of mates per male or per female (Fisher 1930) or the ratio of sexually reproductive males to receptive females (Emlen and Oring 1977). However, descriptions of behavioral interactions between males and females demonstrate that sex ratio or the number of observed copulations may not be an accurate indicator of the mating system (Kokko and Monaghan 2001, Kokko and Johnstone 2002, Simmons and Kvarnemo 2006).

Mating systems are directly related to the intensity of sexual selection (Emlen and Oring 1977, Andersson 1994). With the current use of genetic methods to evaluate mating systems, the relationship between mating success and offspring production can elucidate the relative strength of sexual selection in males and females. Recently, Bateman's gradients have emerged as a method that allows the comparison of intensities of sexual selection across species and taxa (Jones et al. 2002, Becher and Magurran 2003). The relationship can be estimated by calculating the slope of a regression line relating fecundity (number of offspring produced) to mating success (number of mates). A nonzero Bateman's gradient supports that sexual selection is operating in the precopulatory phase of sexual selection (Jones 2009).

These gradients can also be compared between the sexes within a species to assess the opportunity for sexual selection and determine the mating system. For example, if males have a steep Bateman's gradient and females have a shallow gradient, then sexual selection will be stronger in males, and the mating system is likely to be polygynous. If females have a steep Bateman's gradient and males a shallow gradient, then sexual selection tends to be stronger in females, and the mating system is predicted to be polyandrous. If both sexes have Bateman's gradients close to zero, the mating system may be monogamous. If both sexes have steep Bateman's gradients, they may both compete for mates, and the mating system may be polygynandrous.

Due to limited data on genetic outcomes of mating behaviors, polygyny was historically recognized as the predominant mating system in mammals (Krebs and Davies 1993, Birkhead 2000, Storz et al. 2001). Redefining mammalian mating systems as polygynandrous, however, is becoming more common and is currently recognized in 133 species, 33 families, and nine orders (Wolff and Macdonald 2004). Recently, through genetic analyses, a variety of species previously classified as polygynous have been reclassified as polygynandrous, including white-tailed deer (*Odocoileus virginianus*: DeYoung et al. 2009), roe deer (*Capreolus capreolus*: Vanpé et al. 2009), spotted-tailed quolls (*Dasyurus maculate*: Glen et al. 2009), multimammate rats (*Mastomys natalensis*: Kennis et al. 2008), and raccoons (*Procyon lotor*: Nielsen and Nielsen 2007). Thus, continued detailed genetic investigations of mating systems may indicate that polygynandry is the rule rather than the exception (McEachern et al. 2009).

Polygynous and polygynandrous mating systems have different evolutionary consequences. Relative to polygynandrous, polygynous mating systems increase genetic relatedness within groups and genetic differentiation among groups (Chesser et al. 1993, Nunney 1993), reduce effective population size by decreasing the number of males represented in the gene pool (Sugg and Chesser 1994), and affect genetic variability and evolutionary potential (Valenzuela 2000). Furthermore, differences in genetic structure of the population can have important implications for our understanding of population viability, genetic microevolution (Sugg et al. 1996, Parker and Waite 1997), and the intensity of sexual selection (Reynolds 1996) since variance in reproductive success is an important variable in the strength of sexual selection (Wade 1979, Wade and Arnold 1980). Although male reproductive success is typically constrained by the number of mates obtained (Bateman 1948), the link between mating tactics and reproductive success is more imperceptible in females and not easily observable (Jennions and Petrie 1997, 2000).

Ground-dwelling sciurids show a continuum of mating systems from monogamous (e.g., Olympic marmot, *Marmota olympus*: Allainé 2000) to highly polygynandrous (e.g., yellow-pine chipmunk, *Tamias amoenus*: Schulte-Hostedde et al. 2002, 2004). These mating systems typically correlate with the social organization of the species. Ground-dwelling sciurids are also known for their continuum of social organization (Armitage 1981, Michener 1983; 1984, Blumstein and Armitage 1998), from solitary individuals (e.g., woodchucks, *M. monax*) to groups of individuals of different ages and sexes that share space and have markedly overlapping home ranges, and demonstrate cohesive behaviors such as communication (e.g., black-tailed prairie dogs, *Cynomys ludovicianus*). Sociality in ground squirrels has been correlated with a large body size, habitats with short growing or active seasons with adequate resources, a long period of adult/juvenile overlap, and high levels of predation; the effects of these characteristics are clearly seen in the specialized anti-predator behaviors of these animals (e.g., vigilance and alarm calling; Armitage 1981, Michener 1984).

An index of sociality was created by Armitage (1981) using a multivariate analysis of life history traits for 18

species of ground-dwelling sciurids. This sociality index has five categories: rank 1: essentially solitary individuals (e.g., Franklin's ground squirrel, *Poliocitellus franklinii*), rank 2: species that aggregate yet live individually in a favorable habitat (e.g., thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*), rank 3: a male defends a set of individual females within his territory (e.g., arctic ground squirrel, *Urocitellus parryii*), rank 4: a male defends a harem where females share burrows (e.g., yellow-bellied marmots, *Marmota flaviventris*), and rank 5: multi-harem colonies (e.g., black-tailed prairie dogs; Table 1). This model predicts that no species with a mean minimum adult weight <600 g and a total active season of 5 months or greater will be social. The model has been modified to include the active period of adult and juvenile overlap and again high social indices are seen in ground squirrels where the coincidence of activity is greater than 70% between adult and juvenile ground squirrels (Michener 1984). Thus, this model suggests that social behaviors have evolved as a way to minimize aggressive and competitive interactions due to the timing and sequencing of the annual activity cycle (Michener 1984). More recent models focus on the consequences of social complexity rather than on the evolution of complexity and the life history traits needed to increase the number of potential roles that individuals can play within the social network (Blumstein and Armitage 1998).

Most ground squirrel species are classified with a social index of rank 2 or 3 (species that aggregate yet live individually in a favorable habitat or a male defends a set of individual females) and as polygynous (e.g., thirteen-lined ground squirrel; Richardson's ground squirrel, *U. richardsonii*; arctic ground squirrel; Idaho ground squirrel, *U. brunneus*; and California ground squirrel, *Otospermophilus beecheyi*; reviewed in Waterman 2007). However, due to the introduction of genetic and computational methods, several species have been reclassified as polygynandrous due to the high occurrence of multiple paternity in litters (e.g., Columbian ground squirrels, *U. columbianus*; Raveh et al. 2010; Table 1). Furthermore, the detection of multiple mating by females is expected to increase due to new genetic analyses of mating systems (Zeh and Zeh 2001). The established social organization models of ground-dwelling sciurids can be used to make predictions about the mating system and reproductive ecology for a species.

Discrepancies between the classification of social organization and mating system exist for some species. Based on the model incorporating life history traits, round-tailed ground squirrels, *Xerospermophilus tereticaudus*, a small-bodied ground squirrel with a long active season, were predicted to be a rank 2 species that aggregates yet lives individually in a favorable habitat. However, based on behavioral and spatial observations in the field (Dunford 1977a), round-tailed ground squirrels were classified as a rank 4 species having a semi-colonial population structure with a polygynous mating system (Dunford 1977a, Waterman 2007) and thus were considered to be an outlier in the social organization model. The opportunity is high for multiple mating by both sexes of round-tailed ground squirrels given a long breeding season (personal observation), a relatively large average litter size (x=6.5 young, range 1–12; Reynolds and Turkowski 1972), overall high densities of individuals (range 5.3–40 individuals/ha; Drabek 1970, Dunford 1977b), and a predicted low level of sociality (Armitage 1981). Based on these ecological factors, we expected to find a high degree of multiple mating by both males and females in the genetic expression of the mating system as seen in other ground squirrel species with a similar sociality model rank (rank 2, e.g., thirteen-lined ground squirrels).

In this study, we describe the overall genetic mating system of round-tailed ground squirrels in two ways. We used microsatellite DNA markers to determine the patterns of parentage with direct and indirect methods; we also calculated paternity in litters with more than three offspring where mothers were known (direct paternity) and calculated the average relatedness in all litters with more than two offspring (indirect paternity). We compared morphological variables (e.g., body mass, left hind foot length, femur length) and the timing of reproduction to the number of offspring produced to explain differences in reproductive success. We calculated the opportunity of selection (I) and opportunity for sexual selection (I_s) for each sex and Bateman's gradient (β_{ss}) to assess the intensity of sexual selection. Lastly, we evaluate the ground squirrel social organization models and reproductive ecology variables including the genetic mating system in round-tailed ground squirrels and among sciurid species.

Materials and methods

Study area and sampling

We studied round-tailed ground squirrels at Casa Grande Ruins National Monument in Coolidge, Pinal County, Arizona, from January 2004 to June 2007. Round-tailed ground squirrels are small, non-sexually dimorphic, non-territorial ground squirrels that inhabit the desert areas of southwestern USA and northwestern Mexico (Hall 1981). They hibernate for a portion of the year, emerging from their burrow in late January, and are active until July (Dunford 1975). The breeding season is defined by the presence of scrotal males from mid-February through late April and is influenced by the quantity and temporal distribution of spring rainfall (Neal 1965, Reynolds and

Table 1 A comparison of the reproductive traits of sciurids, including the average percentage of multiple paternity (n = number of litters), the marker type used to make that estimate (A = allozyme, F = DNA fingerprinting, and M = microsatellites), the average number of mates per female, the average litter size at emergence, the breeding season length (measured in days), the estrus length (measured in hours), density (number of individuals/hectare), and a sociality index. Measures of variance were included whenever possible. When estimates of paternity in litters were available from multiple genetic techniques, we used the estimate from the analysis that had a higher probability of paternity elimination (e.g., microsatellite versus allozyme data). The sociality index is taken from Armitage (1981) for ground sciurids and Koprowski (1998) for tree squirrels

Species	Average percentage of multiple paternity	Marker	Average mates/female	Average litter size	Breeding season length	Estrus length	Density	Sociality index
Ictidomys tridecemlineatus	50% (n=16)	A	2.3±0.9[a,d]–2.46±0.96[a,d]	6.2±1.4–6.8±0.3[a]	12–13	4–5	2.5–18	2
Otospermophilus beecheyi	89% (n=9)	A	6.7±1.4[a,d]	7.1±1.8[a]	48	6.67±3.18	70.4	2
Spermophilus brunneus	Unknown[b]	A	1.3[d]	5.2±1.4	12–13	4–6	2–14[c]	2
Urocitellus beldingi	78% (n=38)	A	2.7–3.3[d]	4.42±2.06	14	4.7±0.3	1.2–304	2
U. columbianus	57.8% (n=147)	M	2.36±0.55[a]–4.35±0.17[d],	2.1±0.09–5.4	21	5–7	11.6–61.7	4
U. parryii	6% (n=16)	F	1.9±0.8[d]	4.1±1.2[a]–6.1	14	6	1.7±0.3	3
U. richardsonii	80% (n=15)	M	2.1±0.1	3.8–5.2	21–35	3.0±2.3	1.4–27	2
Xerospermophilus tereticaudus	55% (n=33)	M	2.5±0.26	4.1±0.38–6.5	12.75±3.6		5.3–40	2
Cynomys gunnisoni	61% (n=21); 77% (n=44)	M	2.05±0.98[a]	2.91±0.14	60	24	20–85	3
C. ludovicianus	3% (n=273)	A	1.39±0.61[a]	3.08±1.06[a]	14–21	<24	10–35	5
C. parvidens	71% (n=14)	M		3.95±0.26			2.5–74	3
Marmota caligata	58% (n=33)	M		3.12[e]				3
M. monax	63% (n=26)	M	1.8	2.6	7–14	18.1±2.1		1
M. marmota	31.4 (n=34); 19.4±3.9 (n=103)	M		3.57±0.48–4.31±0.17				3
Tamiasciurus hudsonicus	71% (n=38)	M	5.8±0.3	3.0±0.1	60	<24	0.3–2	3.25
Sciurus carolinensis	31%[f] (n=5)	F	3.47±2.12[d]	1.8–3.7	60	<8	3–16	3.25

References: *I. tridecemlineatus*: Rongstad 1965, Mitchell 1972, Streuble and Fitzgerald 1978, Schwagmeyer and Wootner 1985, Foltz and Schwagmeyer 1989, Schwagmeyer 1989; *O. beecheyi*: Boellstorff et al. 1994; *Spermophilus brunneus*: Sherman 1989; *Urocitellus beldingi*: Sauer, 1976, Morton and Sherman 1978, Hanken and Sherman 1981, Holekamp 1983, Sherman and Morton 1979; *U. columbianus*: Shaw 1920, Murie and Harris 1982, Dobson and Kjelgaard 1985, Elliott and Flinders 1991, Murie 1995, Raveh et al. 2010; *U. parryii*: Carl 1971, Hubbs and Boonstra 1997, Lacey et al. 1997a; *U. richardsonii*: Wehrell 1973, Michener and Michener 1973, Michener 1980, Davis, 1982, Hare 2004; *X. tereticaudus*: Drabek 1970, Reynolds and Turkowski 1972, Dunford 1977b; *Cynomys gunnisoni*: Travis et al. 1996, Hoogland 1997; 1999, Haynie et al. 2003; *C. ludoviciaes*: Hoogland 1995; *C. parvidens*: Haynie et al. 2003; *M. caligata*: Barash 1989a, b, Kyle et al. 2007; *M. monax*: Grizzell 1955, Snyder and Christian 1960, Sinha Hikim et al. 1991, Maher and Duron 2010; *M. Marmota*: Allainé et al. 1998, Goossens et al. 1998, Cohas et al. 2008; *S. carolinensis*: Brown and Yeager 1945, Thompson, 1977, Koprowski 1994, David-Gray et al. 1998; *Tamiasciurus hudsonicus*: Gurnell 1987, Lane et al. 2008

[a] SD

[b] A total of 66–100% of juveniles were sired by the last male to guard a female (Sherman 1989)

[c] Based on female nest burrow density

[d] From observation in the field

[e] Calculated from Table 1 (Kyle et al. 2007)

[f] Estimated

Turkowski 1972). Based on spatial and behavioral observations, round-tailed ground squirrels are believed to form a female-based, semi-colonial population structure through the male-biased dispersal of adults in February and juveniles in July (Drabek 1970, 1973; Dunford 1977a).

Creosote bushes (*Larrea tridentata*) dominated the landscape with occasional barrel (*Opuntia* sp.), saguaro (*Cereus giganteus*), and planted ornamental cacti and with trees around a visitors' center and picnic area. Annual precipitation averaged at 225.9 ± 33.1 mm and average daily temperatures ranged between $-4.7 \pm 1.06°C$ and $46.0 \pm 0.95°C$ from 2004 to 2007 (ncdc.noaa.gov). However, 2007 had an extreme drought with a late onset of spring rainfall.

We used Sherman live traps (H.B. Sherman Traps, Inc, Tallahassee, FL, USA) baited with sunflower seeds and/or peanut butter to trap adult squirrels at burrows during the day. We immobilized the squirrels with a cloth handling cone (Koprowski 2002) and released all individuals at the point of capture. Males emerge on average 2 weeks before females; we attempted to capture all individuals within 2 weeks of emergence from hibernation. Upon initial capture, we determined the sex, age class (juvenile <6 months, sub-adult 6–12 months, adult >12 months), and reproductive condition. To determine reproductive condition, we visually inspected the testis position (abdominal, inguinal, or scrotal) for males and visually inspected the teats to assess the degree of distension, alopecia, and pigmentation (Larsen and Taber 1980) and inspected the vulva to determine swelling indicative of estrus for females. We measured body mass (± 5 g) with a Pesola spring scale (Baar, Switzerland), left hind foot length (± 1 mm), femur length (± 1 mm), and testes length (± 1 mm, after the testis had fully descended). We marked the individuals with a unique freeze mark (Rood and Nellis 1980, Koprowski 1996) and hair dye (Clairol Balsam Lasting Color, True Black #618, Procter & Gamble, Cincinnati, OH, USA) for permanent and immediate identification in the field. We used surgical scissors to collect a 4-mm^2 tissue sample from the tail tip upon initial capture for genetic analysis. We stored the tissue samples in 1 mL DMSO buffer solution at $-20°C$ upon exit from the field.

We recorded litter size at emergence aboveground. To ensure that juveniles could be assigned to a litter, we captured individuals by trapping at the target burrow with Sherman live traps and with noosing (Medica et al. 1971, Lacey et al. 1997b, Larrucea and Brussard 2007). To noose animals, we placed a loop of polyester string over burrow entrances and sat 4 m away. When a juvenile came aboveground, we rapidly tightened the noose around the abdomen. We are confident that we captured >90% of littermates from each litter based upon the litter size counts at the first appearance of juveniles aboveground. The University of Arizona Institutional Animal Care and Use Committee (IACUC number 07-026, 04-009) approved the trapping and handling procedures and we obtained trapping permits from the Arizona Game and Fish Department and National Park Service.

DNA extraction and genotyping

We used standard phenol–chloroform methods (Sambrook and Russell 2001) with proteinase K and a Tris-based cell lysis buffer to extract genomic DNA. We amplified seven polymorphic microsatellite DNA loci from other closely related species of ground squirrel (IGS-1, B-109, B-126, GS-12, GS-14, GS-25, GS-26; May et al. 1997, Stevens et al. 1997, Garner et al. 2005) with GoTaq polymerase (Promega Corp, Madison, WI, USA), with primer sets including a fluorescently labeled forward primer (6-FAM or HEX, IDT DNA Technologies, IL, USA) and an unlabelled reverse primer (Table 1). We conducted PCR amplifications in 25-µl volumes containing 50 ng genomic DNA, 1 µM each of fluorescently and non-fluorescently labeled primer, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$, 5 ng BSA, 1X clear flexibuffer, 1 U of GoTaq polymerase, and 8.8 µl of PCR water. The thermal profile consisted of a denaturation cycle at 94°C (4 min), 35 cycles of 94°C (30 s) denaturation, 51°C (30 s) annealing, a 72°C (30 s) elongation, and a final extension at 72°C (5 min). We visualized the PCR products on a 2% agarose gel to detect positive PCR. We performed post-PCR mixing before visualization on an ABI 3130 (Applied Biosystems, Foster City, CA, USA). The genotypes were visualized with GENOTYPER software (v 3.7, Applied Biosystems, Foster City, CA, USA). We scored the chromatogram data twice with two different observers to alleviate scoring errors. We excluded individuals typed at fewer than five loci.

Paternity analyses

A direct analysis of paternity was possible because the suspected mothers were captured and identified at the burrow. For the indirect analysis of paternity, we calculated relatedness among offspring pairs within a litter to determine the average relatedness within a litter. We used GENEPOP 4.0 (Rousset 2008) to assess the characteristics of microsatellites, and test Hardy–Weinberg equilibrium, null allele frequency, and polymorphic information content (PIC), which refers to the value of a marker for detecting polymorphism within a population that depends on a number of detectable alleles and the distribution of their frequency (Rousset 2008; Table 2).

For direct paternity analysis, we incorporated genotypic data into CERVUS 3.03 (Marshall et al. 1998), which calculates the log-likelihood ratio scores to estimate the most likely father of each offspring in each litter based on available genotypes and allele frequencies in the popula-

Table 2 Summary statistics from seven microsatellite loci used for a study of round-tailed ground squirrels (*X. tereticaudus*) at Casa Grande Ruins National Monument, Pinal Co., Arizona (2004–2007) including species where microsatellite originated; repeated core sequences of thymine (T), guanine (G) cytosine (C), and adenine (A); allelic richness of microsatellites; range of allele sizes; observed (H_O) and expected (H_E) heterozygosity and Hardy–Weinberg equilibrium (H–W); and Polymorphic Information Content PIC and null allele frequency

Locus	Species of origin	Core repeats	Allelic richness	Allele size range (bp)	H_O	H_E	H–W	PIC	Null allele frequency
IGS-1	NIGS	TG	10	80–98	0.734	0.744	NS	0.705	0.001
B-109	SIGS	GA	12	209–231	0.834	0.835	NS	0.817	0.002
B-126	SIGS	CA	12	168–190	0.769	0.813	NS	0.788	0.022
GS-12	CGS	TG	6	145–147	0.655	0.623	NS	0.589	0.039
GS-14	CGS	TG	6	224–234	0.712	0.736	NS	0.698	0.014
GS-25	CGS	TG	18	123–151	0.678	0.758	NS	0.725	0.015
GS-26	CGS	TG	4	111–117	0.333	0.332	NS	0.290	0.004

CGS Columbian ground squirrel—*S. columbianus* (Stevens et al. 1997), *NIDGS* Northern Idaho ground squirrel—*S. brunneus brunneus* (May et al. 1997), *SIDGS* Southern Idaho ground squirrel—*S. brunneus endemicus* (Garner et al. 2005)

tion. Other methods may exclude the true parents if genotype errors are present or if the relatives are candidate parents (Marshall et al. 1998, Jones and Ardren 2003, Wagner et al. 2006). We estimated the statistical confidence (delta[Δ]) for critical values at 80% and 95% confidence levels based on a computer simulation of paternity inference with allele frequencies from the study population from CERVUS simulations for 10,000 cycles and assumed that 75% of males in the population had been sampled and 75% of loci had been typed.

The genetic studies of mating systems are often limited by the number and variability of microsatellites used, number of potential parents captured, whether a single parent is known, and the number of litters sampled with more than or equal to three littermates (Bernatchez and Duchesne 2000). The failure to assign paternity with high confidence may simply be due to a deficiency of informative loci. The seven loci that we used to determine paternity had a relatively high level of polymorphism, and PIC, and thus had a high combined probability of detecting multiple paternity within a litter (Table 2).

We used the program ML-RELATE (Kalinowski et al. 2007) to calculate the number of fathers/litter indirectly by calculating the average relatedness of littermates. We imported allele frequencies from GENEPOP and ran each litter individually. We averaged the relatedness of each pair of offspring in each litter to calculate the overall average relatedness within a litter.

Statistical methods

We used regression to test if litter size was related to the number of sires/litter and if body mass, left hind foot length, or femur length was related to reproductive success

and used ANOVA to examine relationships between testes size and reproductive success in males. We calculated a correlation matrix for all reproductive ecology variables and levels of sociality predicted by the sociality models (Armitage 1981, Koprowski 1998) in Table 1. For correlation analysis, we used the median value for all the reported means of reproductive ecology variables. We performed all analyses using JMP-IN (SAS Institute Inc. 2003) and set alpha at 0.05 for statistical significance. All means are reported with their associated standard error.

To determine the opportunity for sexual selection, the value of I for each sex was calculated as the ratio of the variance in offspring numbers, V_O, to the squared average in offspring numbers, O^2, among members of each sex; $I_\male = V_{O\male}/O_\male^2$ and $I_\female = V_{O\female}/O_\female^2$ (Shuster and Wade 2003, Wade and Shuster 2005, Shuster 2008). The difference of the variance in relative fitness between the sexes, $\Delta I = (I_\male - I_\female)$, determines the potential and to what degree the sexes will diverge because fitness variance is proportional to selection intensity. We calculated Bateman's gradient (β_{ss}) as the slope of the least-squares regression of reproductive success (minimum number of offspring) on mating success (minimum number of mates; Andersson and Iwasa 1996, Jones et al. 2005, Jones 2009). We used ANCOVA to examine the interaction between sex and reproductive success to test if there was a difference in Bateman's gradient between the sexes.

Results

We captured and genotyped 351 unique individual round-tailed ground squirrels (98 adult females, 114 adult males, 51 juvenile females, 88 juvenile males) from February 2004

to May 2007. We had over 95% turnover rate for both males and females within our study area over all 4 years. All loci ($n=7$) had a low (<0.039) frequency of null alleles and were in Hardy–Weinberg equilibrium (Table 2). We sampled 38 litters with a mean litter size of 4.1±0.38 young/litter (range, one to 13 young/litter); however, only 31 litters met the criteria of unique mothers with more than or equal to three offspring genotyped at more than or equal to five loci (2007, two litters; 2006, six litters; 2005, nine litters; 2004, 14 litters). The age of the adults is difficult to determine; however, only one marked female of known age was able to produce litters in consecutive years and the litter produced in the second year only consisted of one offspring. Of the 31 litters, 55% showed multiple paternity. The power of CERVUS to assign paternity to individual offspring was moderate to high at high (68%) and relaxed (89%) confidence levels (95% and 80%, respectively). For maternity, CERVUS assigned individual offspring at high (62%) and relaxed (96%) confidence levels (95% and 80%, respectively) and 86 offspring met the 95% average mother–offspring–father exclusion probabilities positive log-likelihood ratios. The offspring not assigned to a father are likely due to non-resident males that were not sampled. Litter size was normally distributed and positively related to the number of fathers/litter (Pearson's correlation coefficient, $N=33$, $p=0.83$, $P<0.001$).

Of the 114 adult males captured, 49% ($n=56$) sired at least one offspring and 27% ($n=15$) sired offspring in multiple litters during a single breeding season. For males that sired more than one litter ($n=15$), 14 males sired young in two litters and one male sired young in three litters. Twenty percent of breeding males ($n=11$) sired offspring in consecutive years; however, no males sired offspring in multiple litters in multiple years. Litters averaged 2.5±0.26 fathers/litter over all litters (range 1–7; Fig. 1).
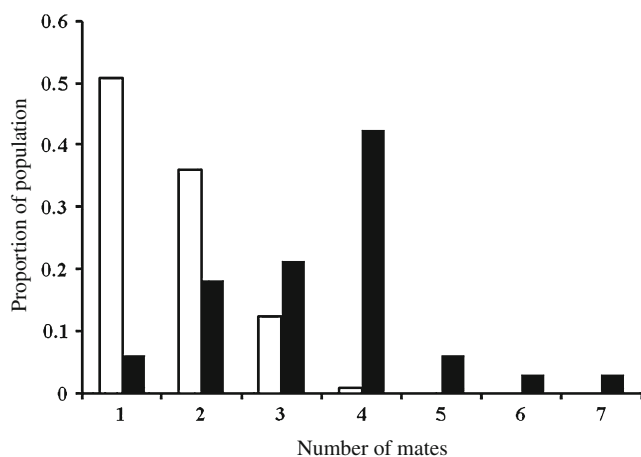
Fig. 1 Number of mates as determined by genotyped offspring for male (*open bars*, $n=56$) and female (*filled bars*, $n=31$) round-tailed ground squirrels (*X. tereticaudus*) at Casa Grande Ruins National Monument, Pinal Co., Arizona (2004–2007)

In females, litter size was not related to body mass ($\overline{X} = 158.2$ g±10.0, $t_{22}=0.17$, $P=0.87$), left hind foot length ($\overline{X} = 32.4$ mm±0.91, $t_{22}=-0.21$, $P=0.84$), femur length ($\overline{X} = 32.1$ mm±0.96, $t_{23}=-1.59$, $P=0.13$), or date of litter emergence ($t_{37}=-0.51$, $P=0.61$). In males, the number of offspring was not related to left hind foot length ($\overline{X} = 33.1$ mm±0.68, $t_{61}=0.74$, $P=0.46$) or femur length ($\overline{X} = 32.6$ mm±0.99, $t_{59}=-0.73$, $P=0.47$); however, body mass showed a negative relationship to the number of offspring produced ($\overline{X} = 161.7±7.7$, $t_{60}=-2.43$, $P=0.02$). Testis size did not differ among males that did not sire young ($\overline{X} = 18.6$ mm±1.5), sired young in one litter ($\overline{X} = 16.8$ mm±1.0), or sired young in multiple litters ($\overline{X} = 18.5$ mm±0.9, $n=44$, ANOVA, $F_{2, 40}=0.0767$, $P=0.96$).

In our indirect analysis of paternity by calculating the average relatedness of littermates using ML-RELATE, 21 litters (68%) had an average relatedness of <0.5, indicating that littermates were less than half-siblings and implying multiple sires. Ten litters (32%) had an average relatedness of <0.25, indicating high levels of multiple paternity (Fig. 2).

Males ($n=56$) had a considerably higher opportunity for sexual selection ($I_s=1.60$) than females ($n=31$, $I_s=0.40$). The difference in the variance between the sexes in relative fitness, $\Delta I=(I_\male-I_\female)$, was 0.99 (Table 3). Bateman's gradient was positive and different from zero in males ($y=1.01x-0.01$, $r=0.92$, $t_{55}=26.44$, $P<0.001$) and females ($y=0.81x+0.26$, $r=0.72$, $t_{30}=-10.2$, $P<0.001$) and was greater in males (1.01±0.04) than females (0.81±0.08: $n=87$, ANCOVA, interaction term, $F_{3, 85}=5.19$, $P=0.024$; Fig. 3).

When we examined the correlation matrix of the predicted levels of sociality to reproductive ecology variables from the sciurid species in Table 1, two relationships emerged. The index of sociality and litter size had a negative relationship ($y=-0.9850454x + 7.242$, $r=0.35$, $t_{13}=-2.65$, $P=0.02$; Fig. 4). The index of sociality and average percentage of multiple paternity in a litter also showed a negative relationship ($y=-0.1455x + 0.9579$, $r=0.34$, $t_{13}=-2.56$, $P=0.02$; Fig. 5).

Discussion

Many small mammals have been classified as polygynous (Waterman 2007), but current molecular techniques often provide additional information about the genetic outcome of mating behaviors that can modify these classifications and influence how we view mammalian mating systems. A diversity of mating systems within ground-dwelling sciurids has been elucidated through the genetic analyses of paternity. Black-tailed prairie dogs (Foltz and Hoogland 1981) and yellow-bellied marmots (Schwartz and Armitage

Fig. 2 Overall average related-
ness with associated SE between
littermates for each litter with
more than two juveniles (*n*=31)
for round-tailed ground squirrels
(*X. tereticaudus*) at Casa Grande
Ruins National Monument,
Pinal Co., Arizona (2004–2007).
An average relatedness of 1
suggests identical twins in a
litter of two young, an average
relatedness of <0.5 suggests at
least two sires for that litter
(*n*=21), and an average
relatedness of <0.25 suggests
three or more sires/litter (*n*=10)
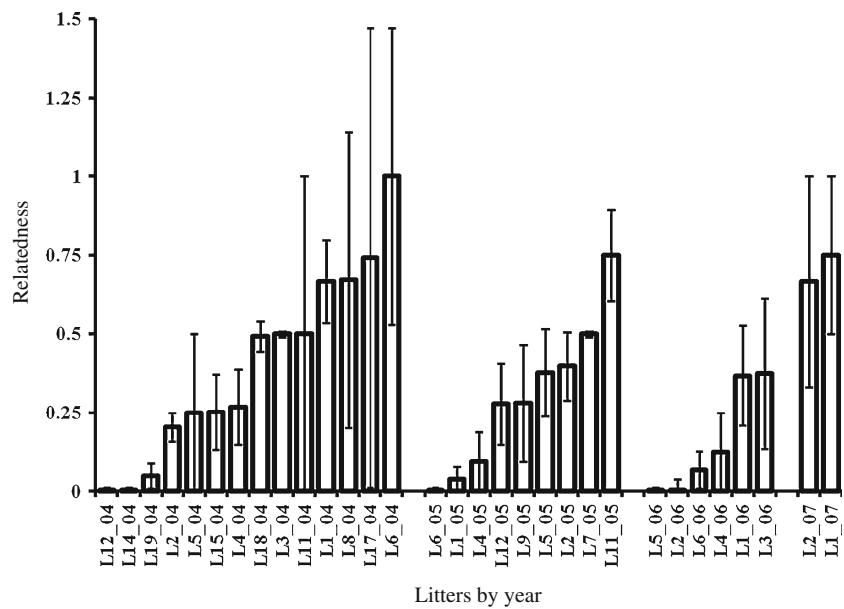
1980) are relatively monogamous and have little to no multiple paternity (<5%) in litters. Extra-pair copulations have also been detected in the monogamous alpine marmot (*M. marmota:* Goossens et al. 1998). California (Boellstorff et al. 1994) and Richardson's (Hare et al. 2004) ground squirrels show high levels of multiple paternity in their litters and Columbian ground squirrels have a polygynandrous mating system where both males and females are mating in multiple (Raveh et al. 2010). In 33 studies of species in the family Sciuridae, 65% of females mated with more than one male (Waterman 2007). Furthermore, sperm competition is known in Belding's (Hanken and Sherman 1981), thirteen-lined (Foltz and Schwagmeyer 1989), and arctic ground squirrels (Lacey et al. 1997a). The prevalence of multiple female mating, sperm competition, and multiple paternity within a litter of ground squirrels suggests that

Table 3 Measure of the opportunity for sexual selection of male and female round-tailed ground squirrels (*X. tereticaudus*) at Casa Grande Ruins National Monument, Pinal Co., Arizona (2004–2007). Opportunity for selection (*I*) is variance in reproductive success (σ_{rs}^2) divided by mean reproductive success ($\overline{X}_{rs}^2$) squared. Opportunity for sexual selection (*I*$_s$) is variance in mating success (σ_{ms}^2) divided by its mean ($\overline{X}_{ms}^2$) squared. Standardized variance in reproductive success (*I*$_s$) describes the opportunity for sexual selection and indicates the maximum strength of sexual selection acting in a population (Wade 1979; Wade and Arnold 1980). Bateman's gradient (β_{ss}) measures the transformation of variation in mating success into variation in Darwinian fitness, as measured by least-squares regression of reproductive success on mating success

Sex	$\overline{X}_{rs}$	σ_{rs}^2	I	$\overline{X}_{ms}$	σ_{ms}^2	I_s	β_{ss} (95% CI)
Male	0.78	0.98	1.60	0.75	0.93	1.65	1.01 (1.05–0.97)
Female	2.50	2.53	0.40	1.56	1.61	0.66	0.81 (0.89–0.73)

females within this taxon play a big role in mate choice and mating strategies.
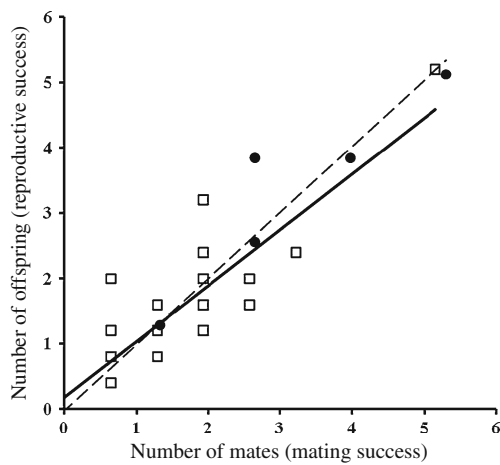
Round-tailed ground squirrels had a polygynandrous mating system during most years when evaluated with molecular genetics, contrary to their previous classification as polygynous (Dunford 1977a). Multiple mating occurred in both sexes and females had sired multiple litters in all years. Males produced offspring with multiple females in 3 of 4 years except during a drought in 2007 when an overall reduction in population size and reproductive levels occurred (Munroe and Koprowski, manuscript in preparation). Of successful males, 27% sired young in multiple litters and 20% were able to sire young in multiple years, suggesting that polygyny was a common mating strategy available to males in our population. The average relatedness of littermates further supports a polygynandry mating system for round-tailed ground squirrels. An indirect paternity analysis showed that 68% of litters had an average relatedness of <0.5, suggesting more than or equal to two sires per litter, and 32% of litters had an average relatedness of <0.25, suggesting even higher levels of multiple paternity. The behavior of mate guarding or prolonged copulation might benefit males and increase their siring success. The observation of copulatory plugs collected in the field (Munroe and Koprowski 2011) also suggests that the high intensity of mate competition among males combined with the high levels of multiple paternity suggests that sperm competition and cryptic female choice may also play a role in this mating system (Koprowski 1992).

Although not previously used to assess ground squirrels, Bateman's gradients standardize the covariance between phenotype and fitness and provide a direct measure of how

Fig. 3 Bateman's gradient for round-tailed ground squirrels (*X. tereticaudus*) at Casa Grande Ruins National Monument, Pinal Co., Arizona (2004–2007). Bateman's gradient is calculated as slope of the least squares regression of relative reproductive success on relative mating success. Females are represented as *open squares* and *solid line*; males are represented by *solid circles* and *dashed line*

mating success relates to the number of offspring produced (Shuster 2009). The sex with a shallow Bateman's gradient is likely to have reproduction limited by the number of offspring, whereas the sex with a steep Bateman's gradient is limited by mating opportunities (Bateman 1948, Arnold and Duvall 1994, Jones 2009). Thus, a trait that increases mating success will have a positive selection differential only if Bateman's gradient is positive. If precopulatory sexual selection arises as a consequence of competition for access to mates, then the currency of sexual selection should be the number of offspring produced or sired. The difference of the variance in relative fitness between the sexes (ΔI) determines whether and to what degree males and females will diverge phenotypically since fitness
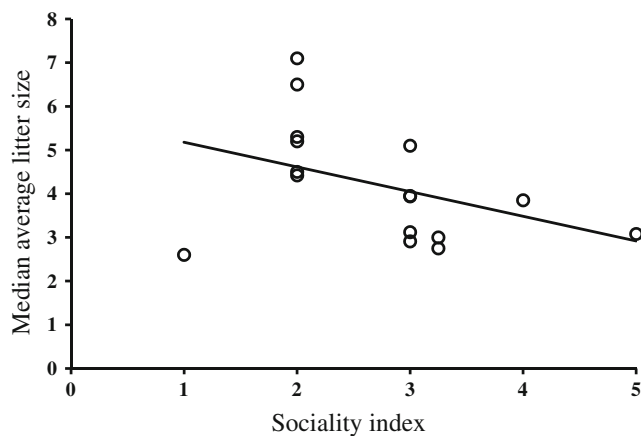
Fig. 4 The relationship between the level of sociality (sociality index, Armitage 1981, Koprowski 1998) and the median average litter size for the sciurid species is listed in Table 1. Sociality in sciurids ranges from solitary individuals (one) to multi-harem colonies (five)
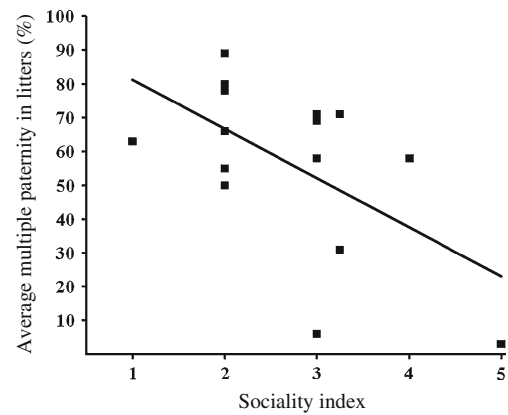
Fig. 5 The relationship between the level of sociality (sociality index, Armitage 1981, Koprowski 1998) and the average percent of multiple paternity in litters for the sciurid species is listed in Table 1. Sociality in sciurids ranges from solitary individuals (one) to multi-harem colonies (five). Paternity analyses were conducted with allozymes, DNA fingerprinting, and microsatellites

variance is proportional to the selection intensity. A positive ΔI for male round-tailed ground squirrel suggests that sexual selection is acting more strongly on males; this may be through pre-copulatory male–male competition for access to females, female mate choice, or post-copulatory mate guarding or sperm competition. Male and female round-tailed ground squirrels have steep positive Bateman's gradients (Fig. 3), suggesting that sexual selection is acting upon both sexes and indicating a polygynandrous mating system (Andersson and Iwasa 1996). Additionally, Bateman's gradient is steeper in males than females, suggesting that males are more limited by their mating opportunities than females. Due to the steep Bateman's gradient, we expect a persistent directional selection on mating success and on any trait correlated with mating success.

Litter size was not associated with female round-tailed ground squirrel's body size, mass, or timing of reproduction. However, females are likely limited by the number of offspring they can produce and care for and possibly the number of sires. An increase in male reproductive success was not associated with increased body size, mass, or testes size. Only 79% of males were captured and weighed within 2 weeks after initial male capture after exiting hibernation; there are several possible explanations of this relationship, including the possibility of lower-quality males that were unable to compete with higher-quality males for mating opportunities However, we assume the negative relationship between male body size and the number of offspring produced to be related to reproductively active males forgoing foraging in order to increase the number of mating opportunities. Furthermore, males may have a single or suite of traits that females are using to make mating decisions, which differentially increases some male mating success.

Among sciurid species, many tactics for increasing reproductive success are observed, and these may correlate with the sociality ranking of each species. A discernable trend exists in male mating order as an advantage in reproductive success in ground squirrels. First, male mating advantage is seen in Belding's (Hanken and Sherman 1981, Sherman 1989), thirteen-lined (Schwagmeyer and Foltz 1990), California (Boellstorff et al. 1994), and arctic ground squirrels (Lacey et al. 1997a). In Columbian ground squirrels, there is a strong first-male advantage in paternity that is tempered with an increased number of mates, indicating that sperm competition plays a significant role (Raveh et al. 2010). Idaho ground squirrels are the exception, exhibiting last-male mating advantage with 66–100% of offspring attributed to the last male to mate with the female (Sherman 1989).

Traditional alternative mating tactics (e.g., satellite males) to increase reproductive success have not been observed in ground squirrels (Raveh et al. 2010). Although paternal care is not common in non-monogamous mammals (Clutton-Brock 1991), male European ground squirrels (*Spermophilus citellus*) with a lower mating success provided parental care through digging and maintenance of natal burrows associated with potential offspring, which led to an increase in litter mass at emergence (Huber et al. 2002). Thus, reproductive success may also be increased through parental care. Additionally, yellow-bellied, alpine, and hoary marmots (*M. caligata*) show behavioral plasticity in their mating systems based on the population density and distribution of individuals (Holmes 1984, Armitage 1986, Kyle et al. 2007).

The established social organization models of ground-dwelling sciurids can be used to make predictions about the mating system and reproductive ecology for a species. The correlation between social structure and litter size (Fig. 4) provides further evidence for the social complexity model of Blumstein and Armitage (1998), where increased social complexity is associated with smaller litter sizes, indicating that sociality has costs in terms of fewer offspring produced per reproductive bout. Sociality has evolved in ground squirrel species with large body size as a way to minimize aggressive and competitive interactions due to the timing and sequencing of the short annual activity cycle (Armitage 1981, Michener 1983; 1984). Therefore, the greater the number of offspring produced, the greater the amount of resource competition during that short active period, and with increased predation, parasite, and disease pressure the greater the predicted level of sociality (Armitage 1981, Michener 1983; 1984). However, an increased number of roles for individuals to fill (e.g., non-reproductive yearling) may provide benefits in future offspring survival.

Based on their social organization rank 2 (aggregates of individuals in a favorable habitat), we expected the round-tailed ground squirrels to have a mating system similar to those of Richardson's, Belding's, thirteen-lined, and Idaho ground squirrels. Our direct paternity analysis estimate of an average of 55% of multiple paternity in litters corresponds to the range of multiple paternity seen in those species (range 50–80%). We would also predict that a first-male advantage would exist in this mating system based on its prevalence in similar species within this social organization and the presence of copulatory plugs (Munroe and Koprowski 2011); however, the number and order of males that each female is copulating with would be necessary to determine male mating strategy.

In relating the sociality models of ground-dwelling sciurids to their reproductive ecology and mating systems, we can see that the social systems not only impact the genetic outcome of the mating system but also affect the genetic structure of the population. When comparing the levels of multiple paternity, there is a significant negative relationship between the level of social organization of a species and the average level of multiple paternity in a litter (Fig. 5). Species that are relatively asocial (rank 1–2) such as woodchucks, Belding's, and Richardson's ground squirrels have higher levels of multiple paternity in their litters, which tend to be larger in size at emergence. Alternatively, highly social species tend to have lower levels of multiple paternity and smaller litter sizes. The variable of litter size obviously impacts the potential level of multiple paternity as smaller litter sizes lack the opportunity to show high levels of multiple paternity. However, this robust relationship is readily observed despite incomplete data (16 of the 45 species of sciurids). Furthermore, several estimates of litter paternity were calculated with allozyme and DNA fingerprinting data, both of which have a reduced ability to differentiate between individuals and reduce the estimate of multiple paternity in litters (*I. tridecemlineatus*: Schwagmeyer and Foltz 1990; *O. beecheyi*: Boellstorff et al. 1994; *S. brunneus*: Sherman 1989; *U. beldingi*: Hanken and Sherman 1981; *U. parryii*: Lacey et al. 1997a; *C. ludovicianus*: Hoogland 1995; *Sciurus carolinensis*: David-Gray et al. 1998). The inclusion of two species of tree squirrels further emphasizes the robustness of the sociality models (Koprowski 1998). Incorporating additional samples from additional sciurid species with recent genetic techniques would most likely increase the explanatory power of the model.

Spatial and temporal distribution of resources and life history tactics determine the social organization, which exerts a direct influence over the mating system (possibly through reproductive skew), subsequent mating opportunities, genetic structure, and ultimately differential individual ecological fitness within patterns of selection within social groups (Ross 2001, Balloux and Lugon-Moulin 2002). However, the patterns of social organization are not only

single functions of ecological factors such as resource distribution, risk of predation, and population density but are products of a subtly interwoven relationship with several evolutionary outcomes such as mating systems. Future studies that manipulate ecological factors or compare populations in habitats of differing quality to determine their effects on sociality and reproductive success will provide insights to the evolution of sciurid mating systems and will advance the understanding of the relationship between ecological patterns and dynamic reproductive strategies (Conrad et al. 2001).

References

Allainé D (2000) Sociality, mating system and reproductive skew in marmots: evidence and hypotheses. Behav Processes 51:21–34

Allainé D, Graziani L, Coulon J (1998) Postweaning mass gain in juvenile Alpine marmots *Marmota marmota*. Oecologia 113:370–376

Andersson M (1994) Sexual selection. Princeton University Press, Princeton

Andersson M, Iwasa Y (1996) Sexual selection. Trends Ecol Evol 11:53–58

Armitage KB (1981) Sociality as a life-history tactic of ground squirrels. Oecologia 48:36–49

Armitage KB (1986) Marmot polygyny revisited: determinants of male and female reproductive strategies. In: Rubenstein DI, Wrangham RW (eds) Ecological aspects of social evolution. Princeton University Press, New Jersey, pp 303–331

Arnold SJ, Duvall D (1994) Animal mating systems: a synthesis based on selection theory. Am Nat 143:317–348

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11:155–165

Barash D (1989a) Marmots: social behavior and ecology. Stanford University Press, California

Barash DP (1989b) Marmots. Stanford University Press, Stanford

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2:349–368

Becher SA, Magurran AE (2003) Multiple mating and reproductive skew in Trinidadian guppies. Proc R Soc Lond B 271:1009–1014

Bernatchez L, Duchesne P (2000) Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? Can J Fish Aquat Sci 57:1–12

Birkhead TR (2000) Promiscuity. Faber & Faber, London

Blumstein DT, Armitage KB (1998) Life history consequences of social complexity: a comparative study of ground-dwelling sciurids. Behav Ecol 9:8–19

Boag DA, Murie JO (1981) Population ecology of Columbian ground squirrels in southwestern Alberta. Can J Zool 59:2230–2240

Boellstorff DE, Owings DH, Penedo MCT, Hersek MJ (1994) Reproductive behaviour and multiple paternity of California ground squirrels. Anim Behav 47:1057–1064

Bradbury JW, Vehrencamp SL (1977) Social-organization and foraging in emballonurid bats. III: mating systems. Behav Ecol Sociobiol 2:1–17

Brown LG, Yeager LE (1945) Fox squirrels and gray squirrels in Illinois. Ill Nat Hist Surv Bull 23:449–536

Carl EA (1971) Population control in arctic ground squirrels. Ecology 52:395–413

Chesser RK, Sugg DW, Rhodes OE, Novak JM, Smith MH (1993) Evolution of mammalian social structure. Acta Theriol 38:163–174

Clutton-Brock TH (1989) Mammalian mating systems. Proc R Soc Lond B 236:339–372

Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, New Jersey

Cohas A, Yoccoz NG, Bonenfant C, Goossens B, Genton C, Galan M, Kempenaers B, Allaine D (2008) The genetic similarity between pair members influences the frequency of extrapair paternity in Alpine marmots. Anim Behav 76:87–95

Conrad KF, Johnston PV, Crossman C, Kempenaers B, Robertson RJ, Wheelwright NT, Boag PT (2001) High levels of extra-pair paternity in an isolated, low-density, island population of tree swallows (*Tachycineta bicolor*). Mol Ecol 10:1301–1308

David-Gray ZK, Gurnell J, Hunt DM (1998) The use of DNA fingerprinting in determining the mating system and reproductive success in a population of introduced gray squirrels, *Sciurus carolinensis* in southern England. In: Steele MA, Merritt JF, Zegers DA (eds) Ecology and evolutionary biology of tree squirrels. Virginia Museum of Natural History, Virginia, pp 43–52

Davis LS (1982) Copulatory behaviour of Richardson's ground squirrels (*Spermophilus richardsonii*) in the wild. Can J Zool 60:2953–2955

DeYoung RW, Demarais S, Gee KL, Honeycutt RL, Hellickson MW, Gonzales RA (2009) Molecular evaluation of the white-tailed deer (*Odocoileus virginianus*) mating system. J Mammal 90:946–953

Dobson FS, Kjelgaard JD (1985) The influence of food resources on life history in Columbian ground squirrels. Can J Zool 63:2105–2109

Drabek CM (1970) Ethoecology of the round-tailed ground squirrel, *Spermophilus tereticaudus*. Dissertation, University of Arizona, Tucson, AZ

Drabek CM (1973) Home range and daily activity of the round-tailed ground squirrel, *Spermophilus tereticaudus neglectus*. Am Midland Nat 89:287–293

Dunford CJ (1975) Density limitation and the social system of round-tailed ground squirrels. Dissertation, University of Arizona, Tucson, AZ

Dunford CJ (1977a) Social system of round-tailed ground squirrels. Anim Behav 25(885):906

Dunford CJ (1977b) Behavioral limitation of round-tailed ground squirrel density. Ecology 58:1254–1268

Elliott CL, Flinders JT (1991) *Spermophilus columbianus*. Mamm Species 372:1–9

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223

Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford

Foltz DW, Hoogland JL (1981) Analysis of the mating system in the black-tailed prairie dog (Cynomys ludovicianus) by likelihood of paternity. J Mammal 62:706–712

Foltz DW, Schwagmeyer PL (1989) Sperm competition in the thirteen-lined ground squirrel: differential fertilization success under field conditions. Am Nat 133:257–265

Garner A, Rachlow JL, Waits LP (2005) Genetic diversity and population divergence in fragmented habitats: conservation of Idaho ground squirrels. Conserv Genet 6:759–774

Glen AS, Cardoso MJ, Dickman CR, Firestone KB (2009) Who's your daddy? Paternity testing reveals promiscuity and multiple paternity in the carnivorous marsupial Dasyurus maculates (Marsupialia: Dasyuridae). Biol J Linn Soc 96:1–7

Goossens B, Graziani L, Waits LP, Farand E, Magnolon S, Coulon J, Bel MC, Taberlet P, Allainé D (1998) Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA micro-satellite analysis. Behav Ecol Sociobiol 43:281–288

Grizzell RA Jr (1955) A study of the southern woodchuck, Marmota monax monax. Amer Midl Nat 53:257–293

Gurnell J (1987) The natural history of squirrels. Facts on File, New York

Hall ER (1981) The mammals of North America, 2nd edn. Wiley, New York

Hanken J, Sherman PW (1981) Multiple paternity in Belding's ground squirrel litters. Science 212:351–353

Hare JF, Todd G, Untereiner WA (2004) Multiple mating results in multiple paternity in Richardson's ground squirrels, Spermophilus richardsonii. Can Field Nat 118:90–94

Haynie ML, Van Den Bussche RA, Hoogland JL, Gilbert DA (2003) Parentage, multiple paternity and breeding success in Gunnison's and Utah prairie dogs. J Mammal 84:1244–1253

Holekamp KE (1983) Proximal mechanisms of natal dispersal in Belding's ground squirrel (Spermophilus beldingi beldingi). Ph. D. dissertation, University of California

Holmes WG (1984) The ecological basis of monogamy in Alaskan hoary marmots. In: Murie JO, Michener GR (eds) The biology of ground-dwelling squirrels: annual cycles, behavioral ecology, and sociality. University of Nebraska Press, Lincoln, pp 250–274

Hoogland JL (1995) The black-tailed prairie dog: social life of a burrowing mammal. University of Chicago Press, Chicago

Hoogland JL (1997) Duration of gestation and lactation for Gunnison's prairie dogs. J Mammal 78:173–180

Hoogland JL (1999) Philopatry, dispersal and social organization of Gunnison's prairie dogs. J Mammal 80:243–251

Hubbs AH, Boonstra R (1997) Population limitation in arctic ground squirrels: effects of food and predation. J Anim Ecol 66:527–541

Huber S, Millesi E, Dittami JP (2002) Paternal effort and its relation to mating success in the European ground squirrel. Anim Behav 63:157–164

Jennions MD, Petrie M (1997) Variation in mate choice: a review of causes and consequences. Biol Rev Camb Philos Soc 72:283–327

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev 75:21–64

Jones AG (2009) On the intensity of selection, the Bateman gradient and the maximum intensity of sexual selection. Evolution 63:1673–1684

Jones AG, Ardren WR (2003) Methods of parentage analyses in natural populations. Mol Ecol 12:2511–2523

Jones AG, Arguello JR, Arnold SJ (2002) Validation of Bateman's principles: a genetic study of mating patterns and sexual selection in newts. Proc R Soc Lond B 269:2533–2539

Jones AG, Rosenqvist G, Berglund A, Avise JC (2005) The measurement of sexual selection using Bateman's principles: an experimental test in the sex-role-reversed pipefish Syngnathus typhle. Integr Comp Biol 45:874–884

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Kennis J, Sluydis V, Leirs H, Pim van Hooft WF (2008) Polyandry and polygyny in an African rodent pest species Mastomys natalensis. Mammalia 72:150–160

Kokko H, Johnstone RA (2002) Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. Proc R Soc Lond B 357:319–330

Kokko H, Monaghan P (2001) Predicting the direction of sexual selection. Ecol Lett 4:159–165

Koprowski JL (1992) Removal of copulatory plugs by female tree squirrels. J Mammal 73:572–576

Koprowski JL (1994) Sciurus carolinensis. Mamm Species 480:1–9

Koprowski JL (1996) Natal philopatry, communal nesting and kinship in fox squirrels and gray squirrels. J Mammal 77:1006–1016

Koprowski JL (1998) Conflict between the sexes: a review of social and mating systems of the tree squirrels. In: Steele MA, Merritt JF, Zegers DA (eds) Ecology and evolutionary biology of tree squirrels. Virginia Museum of Natural History, Virginia, pp 33–42

Koprowski JL (2002) Handling tree squirrels with a safe and efficient restraint. Wildl Soc Bull 30:101–103

Krebs JR, Davies NB (1993) An introduction to behavioural ecology. Blackwell Science, Oxford

Kyle CJ, Karels TJ, Davis CS, Mebs S, Clark B, Strobeck C, Hik DS (2007) Social structure and facultative mating systems of hoary marmots (Marmota caligata). Mol Ecol 16:1245–1255

Lacey EA, Wieczorek JR, Tucker PK (1997a) Male mating behaviour and patterns of sperm precedence in Arctic ground squirrels. Anim Behav 53:767–779

Lacey EA, Braude SH, Wieczorek JR (1997b) Burrow sharing by colonial tuco-tucos. J Mammal 78:556–562

Lane JE, Boutin S, Gunn MR, Slate J, Coltman DW (2008) Female multiple mating and paternity in free-ranging North American red squirrels. Anim Behav 75:1927–1937

Larrucea ES, Brussard PF (2007) A method for capturing pygmy rabbits in summer. J Wildl Manage 71:1016–1018

Larsen JS, Taber RD (1980) Criteria of sex and age. In: Schemnitz SD (ed) Wildlife management techniques manual, 4th edn. The Wildlife Society, Washington, pp 143–202

Loman J, Madsen T, H'akansson T (1988) Increased fitness from multiple matings, and genetic heterogeneity: a model of a possible mechanism. Oikos 52:69–72

Maher CR, Duron M (2010) Mating system and paternity in wood-chucks (Marmota monax). J Mammal 91:628–635

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

May BT, Gavin A, Sherman PW, Korves TM (1997) Characterization of microsatellite loci in the northern Idaho ground squirrel, Spermophilus brunneus. Mol Ecol 6:399–400

McEachern MB, McElreath RL, VanVuren DH, Eadie JM (2009) Another genetically promiscuous 'polygynous' mammal: mating system variation in Neotoma fuscipes. Anim Behav 77:449–455

Medica PA, Hoddenbach GA, Lannom JR (1971) Lizard sampling techniques. Rock Valley Misc Publs 1:1–55

Michener GR (1980) Estrous and gestation periods in Richardson's ground squirrels. J Mammal 61:531–534

Michener GR (1983) Kin identification, matriarchies, and the evolution of sociality in ground-dwelling sciurids. In: Eisenberg JF, Heiman DC (eds) Advances in the study of mammalian behavior. Special publications of the American Society of Mam-

malogy, no. 7. American Society of Mammalogists, Shippensburg, pp 528–572

Michener GR (1984) Age, sex, and species differences in the annual cycles of ground-dwelling sciurids: implications for sociality. In: Murie JO, Michener GR (eds) The biology of ground-dwelling squirrels: annual cycles, behavioral ecology, and sociality. University of Nebraska Press, Lincoln, pp 81–107

Michener GR, Michener DR (1973) Spatial distribution of yearlings in a Richardson's ground squirrel population. Ecology 54:1138–1142

Mitchell RS (1972) Small rodents of the flood plain of the South Platte River at the proposed narrows reservoir site. M.A. thesis, University of Northern Colorado, Greeley, CO

Møller AP, Birkhead TR (1989) Copulation behaviour in mammals: evidence that sperm competition is widespread. Biol J Linn Soc 38:119–131

Morton ML, Sherman PW (1978) Effects of a spring snowstorm on behavior, reproduction, and survival of Belding's ground squirrels. Can J Zool 56:2578–2590

Munroe KE, Koprowski JL (2011) Copulatory plugs of round-tailed ground squirrels (*Xerospermophilus tereticaudus*). Southwestern Nat (in press)

Murie JO (1995) Mating behavior of Columbian ground squirrels. I. Multiple mating by females and multiple paternity. Can J Zool 73:1819–1826

Murie JO, Harris MA (1982) Annual variation of spring emergence and breeding in Columbian ground squirrels (*Spermophilus columbianus*). J Mammal 63:431–439

Neal BJ (1965) Reproductive habits of round-tailed and Harris antelope ground squirrels. J Mammal 46:200–206

Nielsen CLR, Nielsen CK (2007) Multiple paternity and relatedness in southern Illinois raccoons (*Procyon lotor*). J Mammal 88:441–447

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. Evolution 47:1329–1341

Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev 45:525–567

Parker PG, Waite TA (1997) Mating systems, effective population size, and conservation of natural populations. In: Clemmons JR, Buchholz R (eds) Behavioral approaches to conservation in the wild. Cambridge University Press, Cambridge, pp 243–261

Raveh S, Heg D, Dobson FS, Coltman DW, Gorrell JC, Balmer A, Neuhaus P (2010) Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*). Behav Ecol 21:537–547

Reynolds JD (1996) Animal breeding systems. Trends Ecol Evol 11:68–72

Reynolds HG, Turkowski F (1972) Reproductive variations in the round-tailed ground squirrel as related to winter rainfall. J Mammal 53:893–898

Ridley M (1993) Clutch size and mating frequency in Hymenoptera. Am Nat 142:893–910

Rongstad OJ (1965) A life history study of thirteen-lined ground squirrels in southern Wisconsin. J Mammal 46:76–87

Rood JP, Nellis DW (1980) Freeze marking mongooses. J Wildl Manag 44:500–502

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Mol Ecol 10:265–284

Rousset F (2008) GENEPOP 2007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor

SAS Institute Inc (2003) JMP statistics and graphics guide. SAS Institute Inc., Cary

Sauer WC (1976) Control of the Oregon ground squirrel (*Spermophilus beldingi oregonus*). Proc Vertebr Proc Vert Pest Conf 7:99–109

Schulte-Hostedde AI, Millar JS, Gibbs HL (2002) Female-biased sexual size dimorphism in the yellow-pine chipmunk (*Tamias amoenus*): sex-specific patterns of annual reproductive success and survival. Evolution 56:2519–2529

Schulte-Hostedde AI, Millar JS, Gibbs HL (2004) Sexual selection and mating patterns in a mammal with female-biased sexual size dimorphism. Behav Ecol 15:351–356

Schwagmeyer PL, Foltz DW (1990) Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. Behav Ecol Sociobiol 13:1–16

Schwagmeyer PL, Wootner SJ (1985) Mating competition in an asocial ground squirrel (*Spermophilus tridemlineatus*). Behav Ecol 17:291–296

Schwartz OA, Armitage KB (1980) Genetic variation in social mammals: the marmot model. Science 207:665–667

Shaw WT (1920) The cost of a squirrel and squirrel control. Popular Bulletin, State College of Washington Agricultural Experiment Station, 118:1–19

Sherman PW (1989) Mate guarding as paternity insurance in Idaho ground squirrels. Nature 338:418–420

Sherman PW, Morton ML (1979) Four months of the ground squirrel. Nat Hist 88:50–57

Shuster S (2008) Mating systems. In: Jorgensen SE (ed) Encyclopedia of ecology. Elsevier, Oxford, pp 2266–2273

Shuster S (2009) Sexual selection and mating systems. Proc Natl Acad Sci 106:10009–10016

Shuster SM, Wade MJ (2003) Mating systems and strategies. Princeton University Press, Princeton

Simmons LW, Kvarnemo C (2006) Costs of breeding and their effects on the direction of sexual selection. Proc R Soc Lond B 273:465–470

Sinha Hikim AP, Woolf A, Bartke A, Amador AG (1991) The estrous cycle in captive woodchucks (*Marmota monax*). Biol Reprod 44:733–738

Snyder RL, Christian JJ (1960) Reproductive cycle and litter size of the woodchuck. Ecology 41:647–656

Stevens S, Coffin J, Strobeck C (1997) Microsatellite loci in Columbian ground squirrels *Spermophilus columbianus*. Mol Ecol 6:493–495

Storz JF, Bhat HR, Kunz TH (2001) Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. II. Variance in male mating success and effective population size. Evolution 55:1224–1232

Streuble DP, Fitzgerald JP (1978) *Spermophilus tridecemlineatus*. Mamm Species 103:1–5

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. Genetics 137:1147–1155

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. Trends Ecol Evol 11:338–342

Thompson DC (1977) Reproductive behavior of the grey squirrel. Can J Zool 55:1176–1184

Travis SE, Slobodchikoff CN, Kefan P (1996) Social assemblages and mating relationships in prairie dogs: a DNA fingerprint analysis. Behav Ecol 7:95–100

Valenzuela N (2000) Multiple paternity in side-neck turtles *Podocnemis expansa*: evidence from microsatellite DNA data. Mol Ecol 9:99–105

Vanpé C, Kjellander P, Gaillard JM, Cosson JF, Galan M, Hewison AJM (2009) Multiple paternity occurs with low frequency in the territorial roe deer, *Capreolus capreolu*. Biol J Linn Soc 97:128–139

Wade MJ (1979) Sexual selection and variance in reproductive success. Am Nat 114:742–747

Wade MJ, Arnold SJ (1980) The intensity of sexual selection in relation to male sexual behavior, female choice, and sperm precedence. Anim Behav 28:446–461

Wade MJ, Shuster SM (2005) Don't throw Bateman out with the bathwater! Integr Comp Biol 45:945–951

Wagner AP, Creel S, Kalinowski ST (2006) Estimating relatedness and relationships using microsatellite loci with null alleles. Heredity 97:1–10

Waterman JM (2007) Male mating strategies in rodents. In: Wolff JO, Sherman PW (eds) Rodent societies, an ecological and evolutionary perspective. University of Chicago Press, Chicago, pp 17–41

Wehrell S (1973) Social organization and social status in relation to aspects of activity in Richardson's ground squirrel, *Spermophilus richardsonii*. M.S. thesis, University of Alberta, Edmonton, AB

Wolff JO, Macdonald DW (2004) Promiscuous females protect their offspring. Trends Ecol Evol 19:127–134

Zeh JA, Zeh DW (1996) The evolution of polyandry. I. Intragenomic conflict and genetic incompatibility. Proc R Soc Lond B 263:1711–1717

Zeh JA, Zeh DW (1997) The evolution of polyandry II: post-copulatory defences against genetic incompatibility. Proc R Soc Lond B 264:69–75

Zeh JA, Zeh DW (2001) Reproductive mode and the genetic benefits of polyandry. Anim Behav 61:1051–1063