

Mating order and reproductive success in male Columbian ground squirrels (*Urocitellus columbianus*)

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Multiple mating by females is common in many mammalian species, often resulting in mixed paternity litters. In such mating systems, mating order, male age, and male body mass frequently play an important role in determining male reproductive success. We tested for these effects on male reproductive success in Columbian ground squirrels (*Urocitellus columbianus*). The mating activity of estrous females was observed, and the occurrence of sperm precedence was tested using microsatellites to determine paternity in a total of 147 litters (434 offspring), including 110 litters (334 offspring) where the mating position of individual males was determined. Females mated with up to 8 males per litter, whereas paternity analyses revealed that only the first 5 males to mate actually sired offspring. The number of offspring sired significantly decreased with position in the mating sequence, showing a strong first male advantage. The extent of this advantage diminished with an increasing number of male mating partners, indicating that sperm competition plays an important role. A male's position in the females' mating sequences was not consistent within and across seasons, suggesting that individual males did not follow distinct reproductive strategies. Rather, males of intermediate age were more successful than young and old males, when corrected for age effects; heavier males were more likely to mate first. We conclude that males gain the largest part of their seasonal reproductive output from mating first with a female due to a pronounced first male advantage but gain considerable additional fitness from mating with additional, already mated females. *Key words:* Columbian ground squirrel, first male advantage, mating strategy, multiple paternity, senescence, *Urocitellus columbianus*. [*Behav Ecol* 21:537–547 (2010)]

Animals exhibit a diverse array of social systems, including monogamous pairs, harem and lek systems, polyandry, and polygynandry (Krebs and Davies 1996; Wolff and Sherman 2007). Parentage assignment using microsatellite markers has proved invaluable in determining the mating system of different species (Burke and Bruford 1987; Wetton et al. 1987; Kempnaers et al. 1992). Parentage analyses have shown that the interplay between the social system and the mating system may produce a complex array of evolutionary tactics for both males and females (Kleiman 1977; Thornhill and Alcock 1983; Clutton-Brock 1989; Birkhead and Møller 1998).

Mating with numerous females is likely to be advantageous for males, because male reproductive success is typically constrained by the number of mates (Bateman 1948). Although links between multiple mating and increased reproductive success are subtler in females, female multiple mating has been observed in many species (Jennions and Petrie 1997;

Zeh and Zeh 2001). Multiple mating by females selects for more complex male mating strategies, because sperm from different males compete for fertilization ("sperm competition," Parker 1970). Sperm quality and quantity, the timing of mating relative to ovulation, position in the mating sequence, or copulatory plugs may engender differential fertilization success among males (Parker 1970; Hartung and Dewsbury 1978; Baumgardner et al. 1982; Eberhard 1996; Jennions and Petrie 1997; Birkhead and Møller 1998; Simmons 2001; Linn et al. 2007).

Typically, male fertilization success is biased to the first or the last male to mate with the female ("first or last male mating advantage" Birkhead and Møller 1998). The key question has been whether there is any first or last male advantage in a study species (Dziuk 1965; Levine 1967; Parker 1970; Martan and Shepherd 1976; Oglesby et al. 1981; Birkhead and Møller 1992; Simmons and Siva-Jothy 1998; Kraaijeveld-Smit, Ward, Temple-Smith and Paektkau 2002; Pitcher et al. 2003; Kock and Sauer 2007) and how this might come about (e.g., due to variation in sperm investment and mating plugs or due to cryptic female choice, Thornhill and Alcock 1983; Eberhard 1996). As a consequence, the pattern of sperm precedence (nonrandom differential fertilization success of sperm from certain males, independent of the actual sperm numbers:

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Lewis and Austad 1994) may select for one or more male mating strategies (Waterman 2007; Oliveira et al. 2008). Male mating strategies should incorporate both mating frequencies and timing of copulations (e.g., mating order) to maximize reproductive success (Sherman 1989; Schwagmeyer and Foltz 1990; Schwagmeyer and Parker 1990; Lacey et al. 1997; Birkhead and Møller 1998). Access of males to females might also be determined by male age and male body mass (Manno and Dobson 2008; Oliveira et al. 2008). For instance, older males are often more experienced in acquiring access to mates compared with younger males (Clutton-Brock 1988; Danchin et al. 2008), though very old males may experience senescence. Large heavy males may outcompete other males in competition for mates, as found in many taxa or there may be condition-dependent and other alternative mating tactics (Oliveira et al. 2008).

Ground squirrels of the tribe *Marmotini* are highly suitable to assess how different degrees of polygynandry affect male control over reproductive success. There is abundant knowledge of male mating strategies in this mammalian clade (Foltz and Schwagmeyer 1989; Sherman 1989; Schwagmeyer and Parker 1990; Boellstorff et al. 1994; Hoogland 1995; Lacey et al. 1997; Allainé 2000). These species are excellent for studies on mating behavior because females exhibit a very brief receptive phase of up to 12 h in which they typically copulate with several males (e.g., Hanken and Sherman 1981; Schwagmeyer and Parker 1990). This short period allows the documentation of complete mating sequences for estrous females and the identification of mating partners using certain behavioral criteria (e.g., Hanken and Sherman 1981; Schwagmeyer and Parker 1990; Murie 1995; Lacey et al. 1997). We therefore use the term “consort” to refer to behavioral evidence that mating occurred (Lacey et al. 1997) and to the time that a male and female spent together in a burrow. Complementary data on male mating behavior and reproductive success are available for 4 species of ground squirrels: *Ictidomys tridecemlineatus*, 13-lined ground squirrel (Foltz and Schwagmeyer 1989; Schwagmeyer and Foltz 1990), *Urocitellus beldingi*, Belding’s ground squirrel (Hanken and Sherman 1981; Sherman 1989), *Urocitellus parryii plesius*, Arctic ground squirrel (Lacey et al. 1997), and *Urocitellus brunneus*, Idaho ground squirrel (Sherman 1989).

We examined the relationship between mating order and reproductive success of male Columbian ground squirrels. All else being equal, male–male competition, as typically found in all ground squirrel species, should lead to the males distributing themselves evenly over the possible mating positions. However, males are unlikely to be equal: They are likely to differ in quality, for instance, due to differences in male age and male body mass (Schwagmeyer and Parker 1987; Hoogland 1998; Berteaux et al. 1999; Elgar et al. 2003; Adrian et al. 2008). Repeated measures of the mating position of individual males within and across the seasons allowed us to test whether males followed distinct reproductive strategies and assess how reproductive success per mating position contributes to the male’s overall seasonal reproductive output and whether it depends on male characteristics (age and body mass).

Field observations and paternity analyses allowed us to evaluate how a male’s mating position in a female’s mating sequence affected his seasonal reproductive success with multiply mated females. Based on the published information available on related squirrel species, we expected that 1) paternity success should decline with the mating order; 2) males do not use alternative tactics that lead to similar reproductive success (e.g., some males achieve first male advantage in a few litters vs. other males mate later but with more females: the “alternative male strategy hypothesis” Oliveira et al. 2008); and 3) high-quality males achieve higher reproductive success through mating more often overall or through mating more often in the first position.

MATERIALS AND METHODS

Study area

Five neighboring colonies of Columbian ground squirrels (*Urocitellus columbianus*) were studied in the Sheep River Provincial Park, Alberta, Canada (110°W, 50°N, and 1500 m a.s.l., see Appendix Table A1). Columbian ground squirrels are diurnal, colony-living rodents inhabiting subalpine and alpine meadows. Adult males are trapped first from hibernation in mid-April (average: 22.5 April \pm 9.6 standard deviation [SD], range: 12 April–26 May, $n = 71$), followed by adult females (29.0 April \pm 6.6, 11 April–17 May, $n = 154$), yearling males (7.4 May \pm 8.9, 16 April–28 May, $n = 58$), and yearling females (8.5 May \pm 8.5, 21 April–30 May, $n = 90$); see also Murie and Harris (1982). Females mate 4.4 days after emergence from hibernation (adults: 4.4 \pm 2.2 SD days, range: 3–15, $n = 134$; yearlings: 7.0 \pm 2.7 days, range: 5–11, $n = 4$) and are in estrus for about 5–7 h during a single day (Murie and Harris 1982; Murie 1995). Twenty-four days later, females give birth to a litter averaging 3 (1–7) naked and blind juveniles in a specially constructed nest burrow (Murie et al. 1998). The offspring emerge above ground about 27 days postpartum (Murie and Harris 1982).

Sampling of yearlings and adults

Mating behavior and reproduction were recorded from April to mid July 2005–2008. Each colony contained 14–38 adult females and 6–26 adult males (see Appendix Table A1), and females were only observed to mate with males from their own colony (with one exception). The colonies also contained 2–18 yearling females and 5–26 yearling males (Appendix Table A1). Yearling females were observed mating only 5 times, whereas yearling males were never observed mating. Squirrels were caught within the first 2 days of emergence from hibernation with live traps baited with peanut butter (National live traps, Tomahawk, WI, United States; 15 \times 15 \times 48 and 13 \times 13 \times 40 cm). In total, 193 individual females (1 year trapped: 125, 2: 37, 3: 24, and 4: 7) and 186 individual males (1 year trapped: 139, 2: 24, 3: 18, and 4: 4) were successfully trapped, and despite intensive observations during the whole season, no untrapped adults were ever discovered in any colony. Thereafter, animals were trapped weekly and weighted with Pesola spring scales to the nearest 5 g. Individually numbered fingerling fish tags (National Band & Tag Company, Newport, KY, United States; #1) were attached to both ears for permanent identification. In addition, each ground squirrel was uniquely marked with hair dye on their back (Clairol, Hydrience, Procter and Gamble, Stamford, CT, United States; black pearl no. 52) for identification from a distance. We captured unmated, pre-estrous females daily to evaluate their reproductive status until they had mated. The degree of swelling and the openness of the vulva indicate the upcoming day of mating (Murie 1995).

Observations of mating associations

Animals were observed from 3-m-high wooden observation towers, with binoculars. Columbian ground squirrels in our colonies usually mated underground. Mating activity began in the morning between 7:00 and 10:00 lasting until 14:00–17:00 in the afternoon and started with females in estrus leaving their sleeping burrow and roaming around through the colony or vice versa males chasing these females and trying to force them inside a burrow (Murie 1995; Manno et al. 2007). Usually, the male chased the female into a burrow, but sometimes the male entered a burrow where a female in estrus was already present or the male had spent the night together with a female in estrus

(Nestarova A, unpublished data). Estrous females might associate with many males in sequence, accepting some as mating partners, or reject others (e.g., running away or leaving a burrow when being chased in).

If they accepted a male as a mating partner, they would enter a nearby burrow to mate, because copulations usually take place underground. In another population of *U. columbianus* where above-ground copulations were often observed, they lasted on average 35 min (range 1–90 min, Murie 1995). We assumed that underground copulations took place and define a consort as when the estrous female and a male were in the same burrow system and remained there for at least 5 min (average time underground \pm SD: 58.3 ± 32.1 min, range: 8–261 min, and $n = 426$). We used the 5-min criterion because males staying in the burrow for less than this time never sired any offspring according to our paternity analysis. Adults do not normally use burrows during the daytime because they feed outside, except for when hiding from a predator or to retreat from excessive heat.

We are confident that the behavioral criteria allowed us to correctly identify when mating occurred for the following reasons: 1) the minimum consort duration leading to paternity was 15 min, 2) the proportion of offspring sired increased linearly with the relative consort duration for the first and second males to mate, 3) consort duration declined with mating position, indicating that consort duration approximates actual copulation duration (Raveh S, Heg D., Viblanc V. A., Coltman D. W., Gorrell J. C., Balmer A., Dobson F. S., Neuhaus P. unpublished data). Nevertheless, consort duration does not allow us to precisely determine the number or duration of copulations for each male or the interval between successive copulations with different males. Estrus, and therefore mating, was concluded when the focal female increased her feeding activity and avoided or chased potential mating partners away (Murie 1995). A known mating sequence of an estrous female contained all consort partners.

In total, complete mating sequence observations were obtained for 110 litters of 147 litters (75%, see also Table 1: In 37 litters, not all consorts could be determined), so whenever we report individual male mating frequencies and mating positions, these are minimum estimates, assuming the 110 litters are an unbiased sample of all litters.

Sampling of offspring

Nest burrows were identified through observation (for details see, Murie et al. 1998) and marked with colored flags. Females from 3 colonies (1, 2, and 4) were brought to the laboratory where they were kept in polycarbonate cages ($48 \times 27 \times 20$ cm) for 2 days prior to parturition (for more details, see Murie et al. 1998). Within 12 h of parturition, neonates were weighed, sexed, and marked individually by removing a small amount of skin tissue from an outer hind toe or the tail that was later used to determine paternity. Females and their litters were released back into the colony the following day (for details see, Murie et al. 1998), and all females were reaccepted into the colony in their former home range. In the fourth and fifth colonies, tissue for paternity analysis was collected from the ear at juvenile emergence (age: 27 days). However, because we did not sample offspring at birth at colonies 3 and 5, we standardized our analyses among colonies by including only offspring that successfully emerged from their nest burrows at weaning. Offspring were caught within the first 2 days after emergence above ground for the first time, with either unbaited $13 \times 13 \times 40$ -cm National live traps or with multi-capture traps (Murie 1995). Juveniles were marked and weighed, and their sex was determined or confirmed if born in captivity.

Only females with known mating sequences were included in the mating order analyses ($n = 110$ litters), whereas all litters were tested for multiple paternities ($n = 147$ litters), including those of yearling females. Yearling females seldom reproduce successfully, and no mating sequence information was obtained for their litters (5 yearlings mated, of which 3 gave birth and only 2 raised 2 and 1 offspring till weaning, respectively; see also Murie and Harris 1982; Festa-Bianchet and King 1984; Dobson and Murie 1987). In 5 cases, the same individual male mated twice with the female during the mating sequence. Most likely, these males were disturbed during their first consort (or the female successfully escaped), and therefore, these males consorted a second time with the female later during the mating sequence. However, both the first and the second consorts were retained in the analyses, because neither resulted in any offspring sired ($n = 10$ consorts).

Table 1

Number of different sires per litter for the various numbers of mating partners and the percentage of offspring sired per mating position

Number of mating partners	Number of males siring offspring					Percentage offspring sired per mating position					
	<i>n</i> Litters	1	2	3	4	<i>n</i> Offspring	First	Second	Third	Fourth	Fifth
Litters of adult females ($n = 110$) with complete mating sequence											
2	7	5	2	—	—	18	88.9	11.1	—	—	—
3	21	11	9	1	—	63	66.7	22.2	11.1	—	—
4	41	19	16	6	0	119	52.1	27.7	14.3	5.9	—
5	28	9	9	10	0	87	50.6	23.0	17.2	4.6	4.6
6	9	4	3	0	2	30	66.7	10.0	16.7	6.7	0.0
7	3	0	2	1	0	13	38.5	30.8	23.1	0.0	7.7
8	1	0	1	0	0	4	75.0	25.0	0.0	0.0	0.0
Total	110	48	42	18	2	334	57.5	23.1	14.1	3.9	1.5
Litters of adult females ($n = 35$) with incomplete mating											
Unknown	35	12	15	7	1	97					
Litters of yearling females ($n = 2$) with incomplete mating sequence											
Unknown	2	2	0	—	—	3					

Note that the minimum number of mating partners was 2, the maximum number of sires was 4, and males in the sixth to eighth mating positions did not sire any offspring.

Excluded were 1) 6 adult females with 1 litter each, who were experimentally disturbed after their first mating (colony 3, year 2006): DNA genotyping showed that 5 litters were sired by 1 male and that 1 litter was sired by 2 males (20 offspring); 2) 12 females who did not give birth and 26 litters who did not survive until weaning; and 3) 1 litter of 3 offspring where the female was not genotyped (see Appendix Table A1).

Paternal analyses

DNA was extracted using DNeasy Tissue extraction kits (Qiagen, Venlo, The Netherlands), and 13 microsatellite loci were amplified using polymerase chain reaction (PCR). Primer pairs already developed for *U. columbianus* GS12, GS14, GS17, GS20, GS22, GS25, and GS26 (Stevens et al. 1997); *Marmota marmota* BIBL18 (Goossens et al. 1998); MS41 and MS53; (Hanslik and Kruckenhauser 2000); and *Marmota caligata* 2g4, 2h6 (Kyle et al. 2004) and 2h4 GenBank accession no. GQ294553 amplified polymorphic microsatellite loci. PCR conditions and cycling parameters were similar to Kyle et al. (2004) except for an annealing temperature of 54 °C. We tested for deviation from Hardy-Weinberg equilibrium (HWE) at each locus within cohorts, and for linkage disequilibrium between pairs of loci within cohorts using exact tests.

We used CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) for parentage assignment (Appendix Table A2). Maternity was determined by behavioral observation at the nest, whereas paternity was assigned with 95–99% trio confidence (assumed dam–sire–offspring relationship). Maternity was certain for all the offspring born in captivity, as females were held in separate cages. Analyses were conducted for each year and colony separately. The input parameters for the simulation step of CERVUS were 10 000 cycles, 70 candidate fathers, 90% of the population sampled and 1% genotyping error. Consorting, mating males were listed as potential sires when mating behavior was recorded; otherwise, we included all males older than 1 year. Parental assignments were accepted when the offspring had no more than 2 mismatches with both parents.

Statistical analysis

For the statistical analyses, we used SPSS Version 17 (Norusis 2007). The following independent variables were used throughout: year (fixed categorical effect), colony (fixed categorical effect), mating partners (2–8, fixed categorical effect), and mating position (1–8, fixed continuous effect). The mating position effect was analyzed as the number of offspring sired per male with litter size as the offset (Poisson distribution with a log-link function, the offset ensures that the analysis is run on the proportion of offspring sired, Norusis 2007) using generalized estimating equations (GEEs) with individual male identifier as subject (to account for repeated measures per male), mating partners, mating position nested within mating partners, year, and colony as independent variables (the scaling parameter was estimated using the deviance method). Consistency in individual male mating position was analyzed using Spearman rank correlations, both for within-season consistency (mating position litter_{*i*} vs. mating position litter_{*i* + 1}) and between-season consistency (average mating position year_{*t*} vs. average mating position year_{*t* + 1}). Effects of male age, male age squared, and residual body mass (from the age–body mass relationship, see Results) on reproduction were analyzed using GEEs with individual male as subject, corrected for differences due to year and colony effects and using various link functions depending on the type of response variable.

RESULTS

Paternal assignment

In total, 95 males, 113 females, and 434 offspring (Table 1) were successfully genotyped. Of these 434 offspring, 22 offspring were recruited in the subsequent year(s) into the

adult population (6 males and 16 females) and could participate in reproduction, so the total number of unique genotypes was 95 – 6 males + 113 – 16 females + 434 offspring = 620. Note that females that were not mating were not DNA sampled, and immigrant yearling males were not DNA sampled, because they never mated (see Appendix Table A1 for the total number of yearling males and females per colony and year). Our genotyping success rate was 99%, with 80.3% of the ground squirrels genotyped at all 13 loci ($n = 620$). We retained all 13 loci in our analyses as there was no significant deviation from HWE or linkage disequilibrium. All 434 offspring were successfully assigned to both parents: Ninety-five percent of the offspring had 99% trio-confidence, whereas the remaining 2% had 95% trio-confidence, suggesting that our sampling of adults was complete and that unsampled males were unlikely to be the true sires. In total, 412 offspring (95.0%) had zero mismatches with both assigned parents; 21 offspring (4.8%) had one mismatch with an assigned male or female parent; and one offspring had 2 mismatches with the assigned female parent. However, because maternity was certain due to the females giving birth in the laboratory, we assumed that the 2 mismatches in the latter case were due to mutations.

Mating order

We observed the complete mating sequence for 110 litters (334 offspring from 76 individual females, Table 1) of 147 litters from 100 individual females (Table 1), including the mating position and identities of all consorting males (see Table 1 for excluded litters). The number of male sires per litter did not differ between the litters with complete ($n = 110$) versus incomplete mating sequence observations ($n = 37$, Table 1, $\chi^2 = 1.05$, degree of freedom [df] = 3, $P = 0.79$), which suggests that the 110 litters were an unbiased sample of all the litters concerning parentage distribution. Overall, 464 consorts were observed (in 110 litters), with 2–8 mating partners per litter (mean \pm standard error [SE]: 4.2 ± 0.1 , Figure 1a). Only the first 5 males sired offspring, and this distribution was strongly skewed toward males mating first (110 litters; Table 1, Figure 1b). Because we did not observe all consorts a male had, the minimum average (\pm SE) is 4.8 ± 0.3 females per season (range: 1–16, $n = 71$ individual males).

Multiply and singly sired litters

Overall, 85 of 147 litters (57.8%) were sired by more than 1 male (Table 1). Multiply sired litters ($n = 85$) were sired by 2.36 ± 0.55 (mean \pm SD) different males. Of the 110 litters with known mating sequences, 62 litters (56.4%) were multiply sired (Table 1). Singly sired litters ($n = 48$ of 110 litters) were sired by males from the mating positions 1 ($n = 41$), 2 ($n = 4$), and 3 ($n = 3$) or from positions 1 ($n = 32$) and 3 ($n = 3$) when excluding the 13 litters with only 1 offspring.

First male advantage

The mean number of offspring sired by a male strongly declined with his mating position ($n = 110$ litters, Figure 2), with the first male taking the largest share (Table 2: significant effect of mating order) and success more evenly distributed when more males were involved (Table 2: effect of mating partners). Therefore, the first male advantage significantly declined when more male partners were involved, because each successive male (up to the fifth) had a chance to gain some

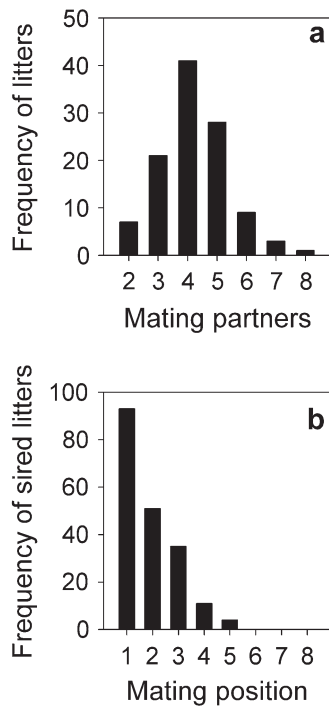


Figure 1
(a) The number of mating partners per litter ($n = 110$) and (b) the frequency of sired litters (at least 1 offspring sired) per male mating position (1 = first to mate, 8 = last to mate, $n = 194$).

reproductive success (GEEs on mating position 1 males only, corrected for year and colony effects; effect of mating partners fitted as a covariate: $\chi^2 = 12.8$, $df = 1$, $n = 110$ of 41 males, $P < 0.001$, coefficient \pm SE: -0.23 ± 0.06).

First males had offspring in 93 of 110 litters (84.5%); second males were successful in 51 of 110 litters (46.4%); third males in 35 of 103 litters (34.0%); fourth males in 11 of 82 litters (13.4%); and fifth males in 4 of 41 (9.8%) litters (Table 1). In 17 litters, the first males did not sire offspring; they might have sired offspring who did not make it to weaning. These 17 cases involved 13 different males, who all appeared fertile because all sired young of other mating events with other females in the same year.

Male reproductive strategies

There was no correlation between the position in the mating order obtained in a litter and the position in the mating order obtained in the subsequent litter for individual males within a season (Figure 3a). Similarly, there was no correlation between the average mating position obtained in a season and the average mating position obtained in the subsequent season (Figure 3b). Of the 24 individual males mating at least 5 times, the observed mating positions did not differ from those expected from the overall probabilities (24 males \times 7 mating positions crosstabulation: likelihood ratio $\chi^2 = 144.6$, $df = 138$, and $P = 0.33$). There was no apparent trade-off between number of matings and proportion of matings achieved in the first position (GLM with logit link, $n = 71$ males; effects of number of matings: $\chi^2 = 0.16$, $df = 1$, and $P = 0.70$). For instance, males having 1–5 matings achieved mating position one 33 times (26%, $n = 126$), 6–10 matings 17 times (21%, $n = 80$), and 11–42 matings 60 times (23%, $n = 258$).

Averaged per breeding season, each male is expected to experience a mean of 0.85 consorts in mating positions 1 and 2,

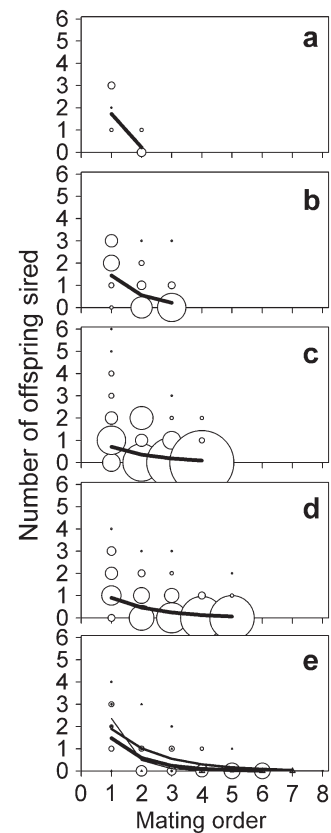


Figure 2
The number of offspring sired per litter ($n = 110$) for each mating position for varying numbers of mating partners (number of litters sampled in brackets): (a) 2 ($n = 7$); (b) 3 ($n = 21$); (c) 4 ($n = 41$); (d) 5 ($n = 28$); (e) 6 (circles, $n = 9$), 7 (triangles up, $n = 3$), and 8 (triangles down, $n = 1$). Overlapping samples are indicated with different symbol sizes (1–36 overlapping data points). Also depicted are the fitted values from the GEE analysis given in Table 2 (corrected for a weighted year and a weighted colony effects).

0.80 consorts in mating position 3, and a rapid decline thereafter (Figure 4a, Friedman Test: $\chi^2 = 161.6$, $df = 7$, $n = 71$ males, and $P < 0.001$). Therefore, seasonal reproductive

Table 2

Effects of the male mating position and the number of mating partners on the male's reproductive success per litter ($n = 464$ events of 71 males, 1–42 events per male)

Parameter	Number of offspring sired		
	df	Wald χ^2	P
Constant	1	89.0	<0.001
Mating order within mating partners	7	150.8	<0.001
Mating partners	6	24.1	0.001
Year	3	32.8	<0.001
Colony	4	12.9	0.012

Number of offspring sired per litter fitted as a Poisson distribution, with litter size as the offset, the scaling parameter was adjusted using the deviance method. Given are results from a GEE with male identifier as subject to account for repeated measures per male ($n = 71$ males) and fixed effects of mating position (covariate, 1–8, nested within mating partners), mating partners (2–8), year (2005–2008) and colony (1–5).

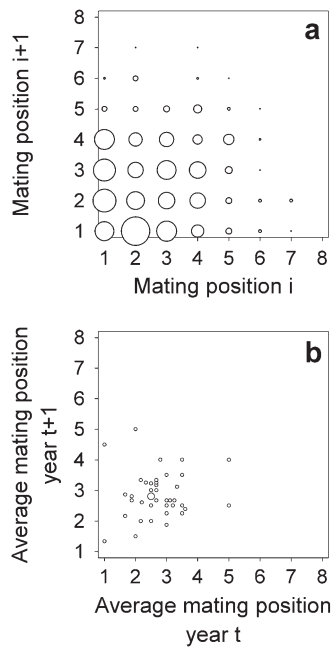


Figure 3 The mating position of individual males was not repeatable. (a) Within-season correlation of male mating position (litter_i vs. next litter_{i+1}, Spearman rank correlation $r_s = 0.033$, $P = 0.54$, $n = 353$). Sample sizes are indicated with different symbol sizes (1–27 overlapping data points). (b) Between-season correlation of male mating position (average of year_t vs. average of next year_{t+1}, $r_s = 0.034$, $P = 0.84$, $n = 40$ males). Sample sizes are indicated with different symbol sizes (1 or 2 overlapping data points). Based on data from 110 litters.

output strongly depends on his siring success from the first mating position (Figure 4b, GEEs $n = 306$ litters mated by 71 males; effects of mating order: $\chi^2 = 193.2$, $df = 1$, $P < 0.001$; year: $\chi^2 = 3.4$, $df = 3$, $P = 0.33$; colony: $\chi^2 = 7.7$, $df = 4$, $P =$

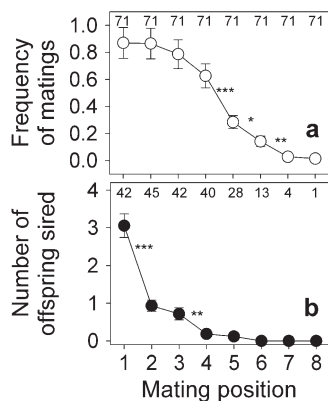


Figure 4 Average seasonal reproductive behavior of individual males (means \pm SEM). (a) Average number of litters males were in mating position 1–8 ($n = 71$ individuals in 110 litters). (b) Average total number of offspring sired per mating position ($n = 1$ –45 individuals per observed mating position, summed over the season in 110 litters). Wilcoxon's signed ranks tests: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, tests in (b) only for individual males observed in both mating position_i and mating position_{i+1} ($n = 31, 29, 29, 24, 5, 0$, and 0, respectively).

0.10). This leads to a tight correlation between seasonal reproductive success and both 1) the number of consorts achieved (Spearman rank correlation, $r_s = 0.70$, $P < 0.001$, $n = 71$) and 2) the number of consortships in the first mating position (Spearman rank correlation, $r_s = 0.77$, $P < 0.001$, $n = 71$ males, data averaged per individual male before analyses). These are all minimum estimates based on the 110 litters with complete mating sequence observations (of 147 litters in total).

Male age and body mass during the mating season

Adult males that were trapped on emergence from hibernation weighed 484 g (± 91 SD, range: 220–645, $n = 70$; missing data were due to most males being trapped 1 to several days after emergence). We retrapped males 358 times on the day they mated with a female (of 594 observed matings) between the 14th of April and 20th of May at which time they weighed 558 g (± 53 SD, range: 425–665, $n = 358$). Comparing the emergence weights of the same individual males (518 g \pm 62 SD) to their weights at first mating (550 g \pm 50 SD, $n = 51$), we found a significant increase of 31 g (paired t -test, $t_{50} = 4.7$, $P < 0.001$) independent of the number of days between these measurements (regression slope $t_1 = 1.44$, $P = 0.16$), suggesting body mass accumulation stopped just before mating. Weights of retrapped males did not change (paired t -test, $t_{89} = -0.58$, $P = 0.57$) between first (564 g \pm 56 SD) and last mating (562 g \pm 50 SD, $n = 90$). Therefore, we used the weight from the nearest trapping date when weight data were missing for a certain mating date to implement the males' body mass. There were 79 cases when males were of known age and body mass, representing 46 males over 1–4 seasons. Males significantly increased in body mass with age in a linear fashion (GEE $n = 79$ of 46 males, effect of age: $\chi^2 = 13.7$, $df = 1$, $P < 0.001$; year: $\chi^2 = 8.8$, $df = 3$, $P = 0.032$; colony: $\chi^2 = 30.5$, $df = 4$, $P < 0.001$), so we used the residual of body mass corrected for age (coefficient \pm SE: 11.371 ± 3.072), year, and colony effects in the subsequent analyses. Note that individuals who remain consistently heavy for their age throughout the years and/or throughout the mating season have positive residuals, whereas individuals who remain consistently light for their age have negative residuals.

Male age, residual body mass, and reproductive strategies

Male age was the most important and significant determinant of male seasonal reproductive output (Figure 5). Males of intermediate age mated most often (Figure 5a), sired more offspring per litter (Figure 5b), and hence had the highest reproductive success (mating frequency \times sired offspring per litter: Figure 5c), compared with younger and older males (Table 3). This effect was apparent in the observed litters ($n = 110$: black circles in Figure 5c) and when the paternity data were included of all litters (i.e., including males not observed to mate at all, $n = 147$: white circles in Figure 5c, Table 3). However, there was no effect of male age on mating order ($\chi^2 = 34.2$, $df = 49$, $P = 0.95$, $n = 378$) even when male age and male age squared were entered into the GEE model presented directly below (both $P > 0.32$).

Relatively larger males were more likely to achieve first mating position ($n = 77$ of 44 males: Figure 6a, probit GEE on the number mated in first mating position/total matings, corrected for year $P = 0.57$ and colony effects $P = 0.98$; effect of residual body mass: $\chi^2 = 10.2$, $df = 1$, $P = 0.001$). Male residual body mass was also positively but weakly related to seasonal reproductive success ($P = 0.053$, Figure 6b, Table 3).

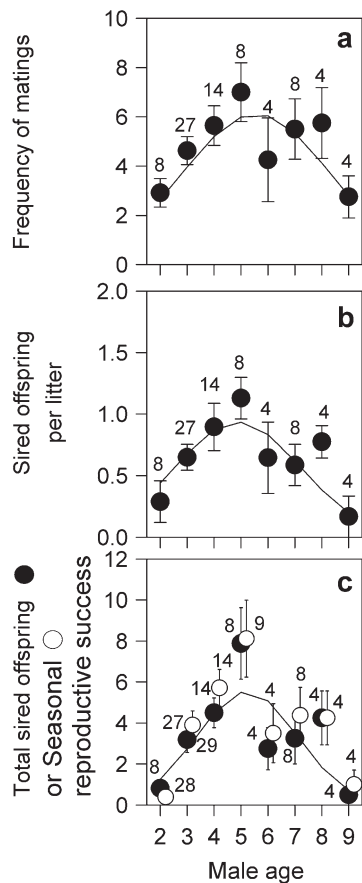


Figure 5
Male age affected seasonal reproductive success (sample sizes indicated on top of the graph): (a) the frequency of matings, (b) the average number of offspring sired per litter, (c) the total number of offspring sired. Black circles: $n = 77$ cases of 44 males, based on paternity in 110 litters; white circles: $n = 100$ of 55 males, based on paternity in 147 litters, that is, including males not mating at all. Quadratic curve fits are from the models depicted in Table 3.

DISCUSSION

Three main results emerged from our mating observations combined with paternity analyses of free-ranging Columbian ground squirrels: 1) as expected, paternity success was not evenly distributed among the males a female mated with, which was due to a strong first male reproductive advantage (first male precedence) with the sixth to eighth mating males siring no offspring at all. However, the first male precedence effect declined with an increasing number of mating partners. 2) Males did not follow alternative mating strategies, where, for example, the mating frequency has to be traded off with the position in the mating order (mating position was inconsistent within and between seasons). 3) Males of intermediate age classes were the most successful in terms of seasonal consortship frequency and reproductive success. These males acquired the first mating position more often, which in itself was affected by relative male body mass (body mass corrected for age effects).

Multiple paternity litters

In our study, we found multiple paternity in 67% of litters, which is a high value for a species with relatively small litters (mean litter size is 3: Dobson and Murie 1987). Overall, many

studies of paternity in polygynandrous mammal species have demonstrated multiply sired litters (e.g., Stockley et al. 1993; Hoogland 1995; Schenk and Kovacs 1995; Bertheaux et al. 1999; Say et al. 1999; Ratkiewicz and Borkowska 2000; Haynie et al. 2003; Kraaijeveld-Smit, Ward, and Temple-Smith 2002; Hare et al. 2004; Solomon et al. 2004; Holleley et al. 2006; Dugdale et al. 2007; Lane et al. 2008).

Most ground squirrel species studied so far show a polygynandrous mating system, where males mate with multiple females in sequence and females mate with multiple males for each litter. Other ground squirrel species show a similar high percentage of multiple paternity within litters often 50% or more (Hanken and Sherman 1981; Foltz and Schwagmeyer 1989; Sherman 1989; Boellstorff et al. 1994), but slightly lower rates of multiple paternity have been reported in Gunnison's prairie dogs (33%, *Cynomys gunnisoni*), whereas a very low occurrence of multiple paternity is found in black-tailed prairie dogs (3% *Cynomys ludovicianus*) and Arctic ground squirrels (9% *Urocitellus parryi*, Hoogland and Foltz 1982; Travis et al. 1996; Lacey et al. 1997). Although 33% of the litters in our study were singly sired, we also found the other extreme: 2 litters containing 4 offspring that were sired by 4 different males. Murie (1995) found a much lower proportion of multiply sired litters (16%) in the same species; however, he speculated that the true value may have been underestimated due to the limited resolving power of allozyme variation (protein electrophoresis) and the low number of protein types used in his study.

Male Columbian ground squirrels appeared to increase their reproductive success by consorting with many females, which often lead to mixed paternity litters. However, it remains unresolved what the female's role is in inducing these multiple paternities (Birkhead and Møller 1992, 1998; Simmons 2001), but the observations suggest that they have substantial control over whom they mate with (by visiting certain males) and they can reject approaching potential mating partners (by running away). Because male Columbian ground squirrels do not provide paternal care and do not perform infanticide, females can only derive genetic benefits or increase the likelihood of successful insemination from mating with certain males (Birkhead and Møller 1992, 1998; Simmons 2001). Genetic benefits from mating multiply can thus only be understood as a mechanism to either increase the likelihood of acquiring the best genes (e.g., when genetic quality assessment is prone to errors) or to increase the genetic diversity of her litter ("genetic bet hedging," e.g., to increase the likelihood that some of her offspring genotypes will maximize offspring fitness in a variable and unpredictable future environment; Birkhead and Møller 1992, 1998; Simmons 2001). The female role and benefit of mating multiply in Columbian ground squirrels need to be tested in the future. The high incidence of multiple paternity suggests there is substantial sexual conflict over parentage in our study species.

Mating order effect and comparison with other related species

Insemination by several males implies that sperm competition (Parker 1970) may play an important role in male Columbian ground squirrel reproductive success. Our results showed a strong first male mating advantage, with relative reproductive success declining nonlinearly with later mating positions. Nevertheless, the relative success of the first male declined significantly with an increasing number of mating partners, because up to the first 5 males were likely to sire at least some offspring. Whether first male precedence is affected by the male, the female, or both, remains to be tested in the future.

Table 3

Effects of male age on male reproductive success per season: the frequency of mating, the average number of offspring sired per litter, and the total reproductive success

Parameter	df	Frequency of mating ($n = 110$ litters)		Sired offspring per litter ($n = 110$ litters)		Total sired offspring ($n = 110$ litters)		Reproductive success ($n = 147$ litters)	
		Wald χ^2	P	Wald χ^2	P	Wald χ^2	P	Wald χ^2	P
n Males		44		44		44		55	
n Total		77		77		77		100	
Constant	1	4.2	0.04	9.4	0.002	10.7	0.001	24.6	<0.001
Male age	1	62.2	<0.001 ^a	10.7	0.001 ^b	28.0	<0.001 ^c	61.2 ^d	<0.001
Male age ²	1	63.4	<0.001 ^c	12.9	0.001 ^f	28.5	<0.001 ^g	53.4 ^h	<0.001
Year	3	12.0	<0.001	2.8	0.52	3.2	0.36	7.6	0.056
Colony	4	155.7	<0.001	2.8	0.59	15.5	0.004	7.1	0.13

Frequency of mating and total sired offspring fitted as a Poisson distribution with a log link, average sired offspring per litter fitted as a normal distribution (Kolmogorov–Smirnov test, $P = 0.29$) with a log link, the scaling parameter was adjusted using the deviance method in each model. Given are results from 4 separate GEEs with male identifier as subject to account for repeated measures per male and the independent variables male age (2–9) and male age squared (covariates) corrected for year (2005–2008) and colony effects (1–5). Residual male body mass was nonsignificant in each of the 4 models and removed ($P = 0.30, 0.41, 0.52, 0.052$, respectively).

Coefficients \pm SE of the male age effects: ^a0.738 \pm 0.094, ^b0.891 \pm 0.272, ^c1.494 \pm 0.283, ^d1.701 \pm 0.275, ^e-0.066 \pm 0.008, ^f-0.091 \pm 0.026, ^g-0.143 \pm 0.027, and ^h-0.161 \pm 0.027.

Our results compare well with other species of Marmotine rodents. In 3 related species, first male reproductive bias has been confirmed: Belding's ground squirrel *U. beldingi* (Hanken and Sherman 1981; Sherman 1989), 13-lined ground squirrel *I. tridecemlineatus* (Schwagmeyer and Foltz 1990) and Arctic ground squirrel *U. parryii* (Lacey et al. 1997). Compared with other ground squirrel species having a first male bias, female Columbian ground squirrels mated on average with more males (see Lacey et al. 1997 for the data on 4 species: range 1–5 males). Additionally, male Columbian ground squirrels mating in the second and later mating posi-

tions had a relatively higher likelihood of siring offspring compared with other species (see Lacey et al. 1997: first male advantage range 60–93%, compared with 57.5% in our study species). For example, males mating in fourth and later positions have virtually no reproductive success in 3 ground squirrel species, despite larger litter sizes (Hanken and Sherman 1981; Foltz and Schwagmeyer 1989; Lacey et al. 1997). Because males up to position 5 sired some offspring, we observed (as expected) the first male precedence effect to decrease with the number of male mating partners by default. However, it would be interesting to show experimentally whether certain males are better in sperm competition than other males, that is, whether some males show a stronger first male precedence effect than other males.

Our findings suggest that males try to gain maximum parentage by mating often and trying to mate in the first mating position. Apparently, monopolizing the first mating position for many females in the colony is not attainable for any male (as indicated by the absence of within- and between-season correlations between the mating positions of individual males), although males of intermediate age were more successful and relatively heavy males outcompeted other males for the first mating position. Male ground squirrels must temporarily vacate their territories to trace spatially dispersed estrous females. Because females are often spatially dispersed, and are not breeding synchronously, they are difficult to defend by the males, and this results into a scramble competition mating system (Murphy 1998) in Columbian ground squirrels. Hence, males ranging widely might be more successful in finding receptive females (Schwagmeyer and Woontner 1986; Schwagmeyer and Parker 1987; Schwagmeyer et al. 1998; Spritzer et al. 2005).

Where patterns of first male sperm precedence are typical for mammalian species, last male sperm precedence is more typical, for example, for insects, birds, and some marsupials: patterns that can be explained in general by the male's and female's reproductive biology and physiology (Birkhead and Møller 1998). The physiological mechanisms most likely leading to first male sperm precedence in mammals very much depend on whether the species is an induced ovulator (i.e., the first male mating induces ovulation) or a spontaneous ovulator (Gomendio et al. 1998; Soulsbury 2010). Unfortunately, we do not know whether Columbian ground squirrels

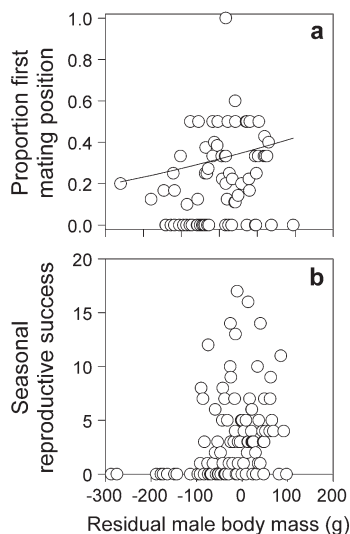


Figure 6

(a) The proportion of litters mated in the first mating position ($n = 77$ of 44 males, based on 110 litters) and (b) seasonal reproductive success of individual males, depending on male residual body mass (mass corrected for age effect, $n = 100$ of 54 males, based on 147 litters and males not mating at all). Also depicted in (a) is the significant GEE regression line (coefficient \pm SE: 0.004 \pm 0.0014, $P = 0.001$), whereas in (b), the relationship was nonsignificant when corrected for age effects ($P = 0.053$).

are induced or spontaneous ovulators, so we cannot discuss the underlying physiological mechanisms leading to first male sperm precedence in this species.

Male age and reproductive success

Columbian ground squirrels are relatively long lived (Neuhaus and Pelletier 2001; Wolff and Sherman 2007), with males and females reaching a maximum age of 9, respectively, 13 years (own observation), compared with other rodents of similar body mass. Male success increased up to 5 years of age. This was not due to 5-year-old males mating more often in the first position (which was independent of age, but depended on body mass, see below) but due to these males mating more often, which also correlates with the likelihood of mating at least once in the first position. Surprisingly, the frequency of consorts, the number of sired offspring per litter, and the total number of sired offspring declined after 5 years of age, perhaps due to senescence. For instance, spermatogenesis is known to decrease with age in several mammalian species (male mutation load, see Ellegren 2007), and this might explain the inability of old males to acquire fertilizations. Alternatively, because female dispersal is limited (Dobson 1982; Murie and Michener 1984; Neuhaus 2006), older males might progressively encounter more and more daughters from previous years as potential mating partners and, due to inbreeding avoidance or depression (e.g., Jennions and Petrie 1997), progressively experience lower reproductive success due to their daughters rejecting them as mating partners.

In our study, the likelihood of acquiring a certain mating position was randomly distributed within and between seasons. At the same time, mating with more females increased the likelihood of mating at least once in the first position. Male Columbian ground squirrels therefore all appear to use the same reproductive tactic of maximizing their reproductive success, and therefore, we found no evidence for alternative reproductive tactics despite many studies to the contrary in other rodents (Schwagmeyer 1985; Schwagmeyer and Wootner 1985; Koprowski 1993; Boellstorff et al. 1994; Travis et al. 1996; Lacey and Wieczorek 2001; Manno and Dobson 2008). Male Columbian ground squirrels often exhibit postcopulatory mate guarding, which may increase chances of siring success (Manno et al. 2007). Mate guarding might limit the number of additional mates the female acquires and therefore increase the male's reproductive success. The behavioral mechanisms behind the observed first male sperm precedence effect in *U. columbianus* will be examined in more detail in a forthcoming study.

We found that relatively heavy males, as measured on the day of consorting, were significantly more often mating in position 1, and accordingly, residual mass tended to positively affect total reproductive success. Results in other species tend to be mixed, for example, effect (Kempnaers et al. 2001; Stapley and Keogh 2005) and no effect (Johnsen et al. 2001; Rakitin et al. 2001). At present, the mechanism leading to this effect remains unknown. For example, better condition males might need less time posthibernation for feeding and accordingly have more time to search for females in estrus. It is important to stress, however, that we determined male body mass and therefore effects on the mating order, for each litter separately. In practice, some individual males increased their body mass over the mating season, some decreased, and others were more variable (Raveh S, personal observation), and overall, we detected no significant change in body mass over the mating season (but all Columbian

ground squirrels quickly increase their body mass thereafter to prepare for hibernation). This suggests that males that happen to be in good condition on the day a female is in estrus may have a good chance to mate in the first position, but it leaves little scope to predict how their body condition and therefore their mating position will be when the next female comes into estrus.

We propose that male–male competition for mating access, depending on the densities of males and females, male home ranges, female roaming behavior during mating (i.e., female mate choice), and male and female characteristics (e.g., age and body mass) should be analyzed in more detail in the future, perhaps on a colony-level basis.

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APPENDIX A

Table A1
Number of yearling and adult males and females sampled in the colonies

Colony	Year	Males ^a			Females ^b		
		Yearling	Adult	Total	Yearling	Adult	Total
1	2007	5	20	25	6	15	21
1	2008	9	16	25	7	17	24
2	2005	8	6	14	11	13	24
2	2006	13	10	23	15	19	34
2	2007	5	15	20	2	22	24
2	2008	8	11	19	8	20	28
3	2006	8	11	19	4	19 ^c	23
3	2007	26	12	38	13	15	28
3	2008	13	14	27	18	15	33
4	2006	5	8	13	5	14	19
5	2008	8	26	34	3	38	41
Total	All	108	149	257	92	207	299

^a Includes 186 individual males.

^b Includes 193 individual females.

^c For 1 adult female, the DNA sample was lost (present only in 2006), and she produced 3 offspring. Because the dam's genotype was missing, her 3 offspring could not be reliably assigned to a candidate male (2 males had both zero mismatches), so her litter was excluded from all analyses.

Table A2
Overview of the microsatellite DNA loci used for parentage assignment, based on the sample of 95 adult males and 113 adult females

Locus	<i>n</i>	Alleles	Het	Hom	H_{Obs}	H_{Exp}	PIC	NE-1P	NE-2P	Null
BIBL18	206	11	167	39	0.811	0.824	0.798	0.525	0.352	+0.0081
GS12	206	7	148	58	0.718	0.685	0.652	0.712	0.527	-0.0330
GS14	202	10	166	36	0.822	0.835	0.810	0.509	0.337	0.007
GS17	185	6	133	52	0.720	0.730	0.684	0.683	0.508	0.0039
GS20B	206	3	50	156	0.242	0.244	0.230	0.970	0.875	0.0186
GS22	204	5	104	100	0.510	0.513	0.459	0.862	0.720	-0.0003
GS25	203	10	165	38	0.813	0.814	0.787	0.544	0.368	0.0016
GS26	207	4	68	139	0.328	0.345	0.318	0.940	0.820	0.0321
Mcal2g4	206	6	141	65	0.684	0.651	0.595	0.766	0.603	-0.0270
Mcal2h4	204	6	138	66	0.676	0.695	0.646	0.722	0.548	0.0141
Mcal2h6	208	4	86	122	0.413	0.441	0.385	0.902	0.782	0.0426
MS41	208	2	81	127	0.389	0.443	0.344	0.902	0.828	0.0629
MS53	205	5	137	68	0.668	0.671	0.602	0.755	0.599	-0.0001

Number of successfully typed adults (n = maximum 208), number of different alleles (alleles), number of heterozygotic (Het), and homozygotic individuals (Hom), observed (H_{Obs}) and expected heterozygosities (H_{Exp}), the polymorphic information content (PIC), nonexclusionary probability of the first (NE-1P) and second parent (NE-2P), and estimated null allele frequencies (Null), respectively, per locus, calculated with the CERVUS software program 3.0.

Combined nonexclusion probability (first parent): 0.0189. Combined nonexclusion probability (second parent): 0.0007.

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