

The mating system of Himalayan marmots as inferred by microsatellite markers

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Abstract

The Himalayan marmot *Marmota himalayana* is widely distributed across the Qinghai–Tibetan Plateau and lives in social groups, yet the mating system of this highly social marmot species is unknown. In this study, the genetic mating system of Himalayan marmots was investigated using microsatellite markers to determine which mating strategies individuals employ. Results revealed that both monogamous and polygamous mating relationships occur in our study population, indicating that the genetic mating system of this marmot species is promiscuity. This study presents the first genetic evidence on the mating system for Himalayan marmots, yet indicates that further studies employing both a genetic and behavioral framework are needed to better understand the social structure and reproductive biology of this marmot species.

Key words: ground squirrels, *Marmota himalayana*, mating system, microsatellites, sociality.

Unlike birds, most of which are considered to be monogamous (a single male and single female mate exclusively with one another) (Cockburn 2006), the majority of mammals employ polygamous mating systems (males or females mate with multiple individuals), while monogamy or promiscuity (both sexes mate multiply) is rare (Clutton-Brock 1989; Lukas and Clutton-Brock 2013). Monogamy is often associated with biparental care and may evolve to mitigate the effects of harsh environments (Wilson 1975; Clutton-Brock 1989; Moss and Moore 2021). Marmot species are hibernators and they usually face harsh environments related to higher altitudes and latitudes, shorter growing seasons, and less vegetation biomass. Consequently, most of them are highly social, living in groups (maybe due to thermoregulatory needs) (Allainé 2000). How this social structure correlates with the genetic mating system is still uncertain. Allainé (2000) states that highly social marmots are primarily genetically monogamous, while Goossens et al. (1998) find extra-pair copulations (EPCs) in Alpine marmots (*Marmota marmota*) (~20% of offspring due to EPCs). Moreover, Schwartz and Armitage (1980) report that yellow-bellied marmots (*M. flaviventris*) have a polygynous mating system, while Kyle et al. (2007) documented facultative mating strategies among hoary marmots *M. caligata*.

The Himalayan marmot *Marmota himalayana* is a large ground squirrel of the genus *Marmota* that can be found widely at elevations of 1,900–5,200 m asl around the Qinghai–Tibetan Plateau (QTP), which is known as “the

roof of the world” with an average elevation of 4,000 m asl (Shrestha 2016; Bai et al. 2019). The QTP is among the most extreme environments in the world and is characterized primarily by low atmospheric oxygen pressure (50–60% of that in lowland regions) and cold climate: The average temperature during the year ranges from 5.6 to 8.8 °C (Wu 2001). The winter is long and cold, and the summer is short and cool with a frost-free period of 100–177 days, resulting in a short growing season for plants and hibernating mammals alike (Xe 1959; Bhandari and Zhang 2019). The hibernation period of this marmot species is up to 6 months (Wang 1992). The Himalayan marmot lives in family groups composed of a resident pair, subordinate adults, yearlings, and juveniles of the year (Bibikow 1996; Allainé 2000); however, the genetic mating system of this highly social marmot species is unknown. Here, we investigated microsatellite variations of a Himalayan marmot population in Zoige in Sichuan Province, China, located in the northeastern part of the QTP, in order to determine their genetic mating system.

Materials and Methods

Sample collection

Himalayan marmots were live captured with wire cage traps around Duoma (103.01°E, 33.5°N, ~3,500 m asl.), a village approximately 8.5 km southwest of the town of Ruoergai County in Sichuan Province, China, during the marmots’ active period (not in hibernation, April to October) in 2020.

To facilitate the identification of the recaptured individuals, captured marmots were marked with uniquely numbered metal ear tags. All possible fathers and mothers were sampled as best as we could. The groups were trapped adjacent to each other, and at least 6 individuals were captured from each group. A total of 77 Himalayan marmots (including 18 juveniles: born in 2020, 22 subadults: born in 2019, and 37 adults) from 9 groups were captured (Table S1). We determined sex by examining anogenital distance or the presence of testes or distended nipples during breeding and lactation, respectively. We estimated age with body mass (juveniles: <2.8 kg generally, as juveniles would gain weight quickly from April to October), body length (juveniles: <30 cm generally), the growth of molars (juveniles: the development of molar teeth is not complete; Yang and Chen 1993), together with continuous observation data from 2018 to 2020 employing a mark-recapture method. We collected small pieces of tail samples from all captured animals and stored in ethanol for DNA extraction.

DNA extraction and microsatellite analysis of parentage

Tail samples were kept overnight with proteinase K, and genomic DNA was extracted using the TSINGKE DNA purification kit (TSINGKE, Beijing, China). The quality and integrity of the extracted DNA were checked by measuring the A260/A280 ratio using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and by agarose gel electrophoresis. Primers for 19 polymorphic microsatellite loci fluorescently labeled with either FAM, HEX, or TAMRA dyes were synthesized by TSINGKE Biological Technology (TSINGKE, Beijing, China) (Table 1).

Polymerase chain reaction (PCR) amplification was performed in a 20 μ L reaction including 17 μ L of Goldenstar™

MIX (TSINGKE), 1 μ L of forward primer, 1 μ L of reverse primer, and 100 ng of genomic DNA. PCR conditions were as follows: 98 °C for 2 min, then 35 cycles of denaturation at 98 °C for 10 s, annealing at 60 °C for 10 s, and extension at 72 °C for 10 s, followed by 72 °C for 5 min. Amplified products underwent capillary electrophoresis on an ABI 3730 automated sequencer, and genotypes were scored using GENEMAPPER software v4.1 (Applied Biosystems).

Micro-checker software (Van Oosterhout et al. 2004) was used to check for unexpected mutation steps, unusually sized alleles, and null alleles for the dataset. Genepop v4.0 (Raymond and Rousset 1995) was used to test for linkage disequilibrium (LD) and deviations from Hardy–Weinberg equilibrium (HWE). Observed and expected heterozygosity (H_O and H_E , respectively) were estimated in Genalex v6.41 (Peakall and Smouse 2006). The Cervus v3.0 program was used to evaluate exclusion probabilities for possible parentage (Kalinowski et al. 2007) and both adults and subadults were considered as candidate parents. The simulations required for paternity or maternity assignments with Cervus were run with 10,000 cycles, a typing error rate of 0.01, and a proportion of 80% sampled candidate parents as per Heckel and Von Helversen (2003). A male or female was accepted as the parent of a particular offspring only if Cervus assigned an individual as a parent with a confidence level of >95%.

Results

Nineteen microsatellite loci (the combined probability of exclusion was >0.9977 when parents are unknown) were used to analyze the kin relationships among these marmot individuals (Table 1). None of the LD and HWE tests were significant. Checking with micro-checker software revealed that the presence of null alleles had no significant effect on

Table 1 Microsatellite loci selected for parentage determination of Himalayan marmots, and the size range, the number of alleles (Na), the observed (H_O) and expected (H_E) heterozygosity, and the frequency of null alleles ($F(\text{null})$) for each locus in our studied marmot population

Locus	Primer sequences-F	Primer sequences-R	Size range (bp)	Na	H_O	H_E	$F(\text{null})$
HM-1	GAAATAGGCTGGTCCGTG	CATACTTGATAGATGGTGGTG	99–106	6	0.662	0.591	–0.1023
HM-2	ATCCGTCCAATAAAGAAATTC	GTTTCTTGTTGGCTCAGTGGTCAGATG	194–300	6	0.662	0.652	–0.0014
HM-3	CCTGTGTGAGTCCCTGGAGTC	AGCCATTTAGGTTACATCTGC	159–175	6	0.779	0.738	–0.0381
HM-4	TTTTTGGCTAACATAGTGGT	AGTGAAGGCTAAAAGCAGAGT	162–178	5	0.701	0.661	–0.0343
HM-5	CTTGTTCAAGATTTGGCTAT	AATGTCTTGAAAATGGTGT	224–234	6	0.805	0.799	–0.0064
HM-6	ATGGCAGAGAATATAAAATGG	CTGGTGGAACTTGTTAGGAG	155–178	12	0.766	0.738	–0.0227
HM-7	GGAAGACCACAGAGGAACAG	CCTTGAAGAGCAAGAGCATA	130–236	5	0.499	0.514	0.0690
HM-8	TAATATCCCCCAAAGAAGTA	TAGACCTTGCTGTGAAAAAT	191–200	6	0.844	0.785	–0.0433
HM-9	ATGGGACAGAACTCTTGATT	CCTTATAGTTTTACCTCCTCC	213–232	4	0.701	0.698	0.0008
HM-10	CATTGGAAGACAGAAAATACA	CAGTCCTTTGAAACTTGAGTA	168–183	4	0.649	0.623	–0.0339
HM-11	AATAGCCAGTTC AACCTC	ATGCTAACTTCAGCAACA	162–171	6	0.662	0.652	–0.0014
HM-12	ATGGTCATGGAAGGGAAG	GGCATCTTCACAGTTGATCT	125–137	5	0.610	0.634	0.0117
HM-13	ATTCTCTAGTCGTTAACAAAGAATC	CACCACTGAAAACATACATACAGTG	191–214	6	0.740	0.742	0.0025
HM-14	CTCATGACTATGGCAGCC	AGAACCTTGATTTAGCAGTAG	128–141	8	0.390	0.461	0.0872
HM-15	TTACACCTTCTCTGGCTCC	TCTGAGCGGATTGTCTTTAT	136–153	4	0.597	0.564	–0.0352
HM-16	ATGGAAAAGGTAAAGAGGGG	GGACAAAACATCAACACAGAG	124–137	4	0.558	0.591	0.0202
HM-17	ATTGAGGAGCAGCATCTAGG	TCAGGAAAAGGCAGACCTG	126–136	8	0.844	0.822	–0.0175
HM-18	CAGACTCCCACAGTGACC	CCTGATCTATGTAGGTTCCAT	94–115	6	0.870	0.814	–0.0385
HM-19	CCTGTGTGAGTCCCTGGAGTC	AGCCATTTAGGTTACATCTGC	160–175	4	0.610	0.614	0.0136

Table 2 Parentage determination of Himalayan marmots

Offspring	Maternity	Pair LOD score	Confidence level	Paternity	Pair LOD score	Confidence level
Z2	Z27	8.74E+00	*			
Z4				Z9	8.56E+00	*
Z5				Z9	8.53E+00	*
Z6				Z1	6.51E+00	*
Z7				Z9	6.90E+00	*
Z10				Z70	6.20E+00	*
Z11				Z70	6.32E+00	*
Z12	Z15	7.93E+00	*	Z28	7.74E+00	*
Z21	Z23	9.39E+00	*	Z70	4.22E+00	*
Z22	Z23	1.14E+01	*			
Z24	Z26	6.37E+00	*	Z9	5.95E+00	*
Z25	Z26	6.29E+00	*	Z9	8.17E+00	*
Z39	Z32	5.31E+00	*	Z46	9.41E+00	*
Z42	Z41	9.94E+00	*	Z46	1.24E+00	*
Z43				Z46	3.56E+00	*
Z44				Z46	7.03E+00	*
Z45	Z41	8.61E+00	*	Z46	5.10E+00	*
Z54	Z55	1.84E+01	*	Z64	1.20E+01	*
Z58	Z59	6.93E+00	*	Z61	1.02E+01	*
Z60	Z59	7.74E+00	*	Z61	1.30E+01	*
Z65	Z55	1.33E+01	*			
Z67	Z69	4.91E+00	*	Z70	1.09E+01	*
Z68	Z69	4.85E+00	*	Z71	9.31E+00	*
Z74	Z73	1.75E+01	*			

Asterisks indicate 95% confidence. Pair LOD score: the comparison value of the log likelihood ratio between offspring and candidate parents.

the data for our marmots. The number of alleles for each locus ranged from 4 to 12, and observed heterozygosity for each locus ranged from 0.39 to 0.87 (Table 1). Finally, a total of 15 mother–offspring relationships and 20 father–offspring relationships were detected in this subset of individuals (Table 2).

Notably, 6 monogamous matings were observed in this population (Table 3), along with 2 polygynous (a male mate with more than 1 female and produces offspring with each female) and 3 polyandrous (a female mates with more than 1 male and produces offspring with each male) relationships (Table 3). Moreover, EPCs occurred both within groups and with neighboring individuals. For example, individual Z69 mated with 2 males from the same group: Z70 and Z71, whereas Z46 mated with 2 females from different groups: Z41 and Z32 (Tables 3 and S1).

Discussion

Using molecular genetic methods, we found that nearly half of male–female matings in our study population were genetically polygamous (2 polygyny, 3 polyandry) and that EPCs occurred both among group members and neighbors, indicating that the mating strategy in the Himalayan marmot was flexible. These findings reveal that the genetic mating system of this highly social marmot species is promiscuous rather than primarily monogamous. Further study with

observational data on social structure will provide further insight into the mating system of the Himalayan marmot both socially and genetically.

Intraspecific variation in mating systems has been recognized across taxa, with mating systems shifting from polygyny to monogamy (socially and/or genetically) even within the same population when food resources decline (Maher and Burger 2011; Streatfeild et al. 2011). Previous studies have suggested that hoary marmots (*M. caligata*) are polygynous in their southern range (Barash 1989) yet monogamous in northern parts of their distribution (Holmes 1984). That said, Kyle et al. (2007) found a mixture of monogamous and polygynous systems within the same population, which was thought to be a consequence of resource limitations such as the distribution of hibernacula in northern regions and also low primary productivity as reflected in aboveground plant biomass. The altitude of marmot population we studied is 3,500 m asl, and the colonization range of Himalayan marmots could reach over 5,000 m asl. Populations of Himalayan marmots from higher altitudes and a harsher environment might employ different mating strategies, with a reduced incidence of EPCs since monogamy may evolve to mitigate the effects of harsh environments. Further investigations of additional populations of Himalayan marmots would clarify the relationships between their mating system and environmental variables such as elevation, temperature, and resource availability.

Table 3 Parent–offspring relationships and mating strategies in the Himalayan marmot population in 2020 in Zoige (103.01°E, 33.5°N) in Sichuan Province, China

Parent-1	Parent-2	Offspring	Mating strategy
Z1	UF	Z6	Monogamy
Z9	Z26	Z24, Z25	Monogamy
Z27	UM	Z2	Monogamy
Z28	Z15	Z12	Monogamy
Z61	Z59	Z58, Z60	Monogamy
Z73	UM	Z74	Monogamy
Z46	Z41	Z42, Z45	Polygyny
	UF	Z43, Z44	
	Z32	Z39	
Z70	Z23	Z21	Polygyny
	Z69	Z67	
	UF	Z10, Z11	
Z23	Z70	Z21	Polyandry
	UM	Z22	
Z55	Z64	Z54	Polyandry
	UM	Z65	
Z69	Z70	Z67	Polyandry
	Z71	Z68	

UF and UM indicate unknown female and unknown male, respectively. The unknown individuals maybe already dead or may live outside the studied social group.

Ethics

The samples used in this study were collected in accordance with the Chinese legal requirements. All animal works were evaluated and approved by Central South University of Forestry and Technology's Institutional Animal Care and Use Committee (protocol no. 2012-018).

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Conflict of Interest statement

The authors declare that they have no competing interests.

Author Contributions

Y.C., C.G., and Z.X. contributed to research design. C.G. and S.Z. contributed to data collection. Y.C., C.G., and S.Z. analyzed the samples. Y.C. and Z.X. discussed the data and wrote the article.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

References

- Allainé D, 2000. Sociality, mating system and reproductive skew in marmots: Evidence and hypotheses. *Behav Proc* 51:21–34.
- Bai L, Liu B, Ji C, et al., 2019. Hypoxic and cold adaptation insights from the Himalayan marmot genome. *iScience* 11:519–530.
- Barash DP, 1989. *Marmots*. Stanford (CA): Stanford University Press.
- Bhandari J, Zhang Y, 2019. Effect of altitude and soil properties on biomass and plant richness in the grasslands of Tibet, China, and Manang District, Nepal. *Ecosphere* 10:e02915.
- Bibikow DI, 1996. *Die Murmeltiere der Welt*. Heidelberg: Spektrum Akademischer Verlag.
- Clutton-Brock TH, 1989. Review lecture: Mammalian mating systems. *Proc R Soc Lond B* 236:339–372.
- Cockburn A, 2006. Prevalence of different modes of parental care in birds. *Proc R Soc Lond B* 273:1375–1383.
- Goossens B, Graziani L, Waits LP, Farand E, Magnolon S et al., 1998. Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA microsatellite analysis. *Behav Ecol Sociobiol* 43:281–288.
- Heckel G, Von Helversen O, 2003. Genetic mating system and the significance of harem associations in the bat *Saccopteryx bilineata*. *Mol Ecol* 12:219–227.
- Holmes WG, 1984. The ecological basis of monogamy in Alaskan hoary marmots. In: Murie JO, Michener GR, editors. *The Biology of Ground-Dwelling Squirrels*. Lincoln (NE): University of Nebraska Press, 250–274.
- 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.
- Kyle CJ, Karels TJ, Davis CS, Mebs S, Clark B et al., 2007. Social structure and facultative mating systems of hoary marmots *Marmota caligata*. *Mol Ecol* 16:1245–1255.
- Lukas D, Clutton-Brock TH, 2013. The evolution of social monogamy in mammals. *Science* 341:526–530.
- Maher CR, Burger JR, 2011. Intraspecific variation in space use, group size, and mating systems of caviomorph rodents. *J Mammal* 92:54–64.
- Moss JB, Moore AJ, 2021. Constrained flexibility of parental cooperation limits adaptive responses to harsh conditions. *Evolution* 75:1835–1849.
- Peakall R, Smouse PE, 2006. Genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6:288–295.
- Raymond M, Rousset F, 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J Heredity* 86:248–249.
- Schwartz OA, Armitage KB, 1980. Genetic variation in social mammals: The marmot model. *Science* 207:665–667.
- Shrestha T, 2016. *Marmota himalayana*. The IUCN Red List of Threatened Species 2016: e.T12826A115106426. <http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T12826A22258911.en>
- Streatfeild CA, Mabry KE, Keane B, Crist TO, Solomon NG, 2011. Intraspecific variability in the social and genetic mating systems of prairie voles, *Microtus ochrogaster*. *Anim Behav* 82:1387–1398.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P, 2004. Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538.
- Wang ZJ, 1992. Ecological observation of *Marmota himalayana* in hibernation period [in Chinese]. *End Dis Bull* 7:51–55.
- Wu T, 2001. The Qinghai-Tibetan plateau: How high do Tibetans live? *High Alt Med Biol* 2:489–499.
- Xe JZ, 1959. *The Qinghai-Tibetan Natural Geography [in Chinese]*. Beijing: Science Press.
- Yang GR, Chen RH, 1993. Age identification of Himalayan marmots of Yunnan population [in Chinese]. *End Dis Bull* 8:65–67.
- Wilson EO, 1975. *Sociobiology*. Cambridge (MA): Harvard University Press.