Zoological Studies **51**(2): 204-212 (2012)

Zoological Studies

Monogamous System in the Taiwan Vole *Microtus kikuchii* Inferred from Microsatellite DNA and Home Ranges

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(Accepted September 14, 2011)

Jung-Sheng Wu, Po-Jen Chiang, and Liang-Kong Lin (2012) Monogamous system in the Taiwan vole *Microtus kikuchii* inferred from microsatellite DNA and home ranges. *Zoological Studies* **51**(2): 204-212. The Taiwan vole *Microtus kikuchii* is considered socially monogamous based on indirect information of captive behaviors and home-range ecology. Genetic components of its mating system were not previously examined. We tested the hypotheses that *M. kikuchii* is both socially and genetically monogamous by combining field information of home ranges with genetic analysis of relationships among individuals. Trapping was conducted in the Hehuan Mt. area of Taroko National Park, central Taiwan, from June 2004 to Aug. 2005. We chose 16 microsatellite loci using primers designed for *M. oeconomus* and *M. montebelli* to amplify *M. kikuchii* DNA. Eleven loci produced clear, polymorphic banding patterns and were used for the genetic analysis. The home-range sizes of adults did not significantly differ between sexes or among seasons. For the 14 social units indicated by overlapping home ranges, 11 (78.6%) were male-female pairs. The other 3 social units involved more than 2 individuals. In two of these, ranges of a male-female pair overlapped ranges of their offspring and other individuals. The genetic analysis revealed that some of the male-female pairs identified by overlapping home ranges did not reproduce. Information based on the home-range data was not powerful enough to identify genetic components of *M. kikuchii*'s mating system and may provide misleading results. A parentage analysis based on microsatellite genotyping revealed litters (with a total 31 of offspring) sired by 18 males and 20 females. The only 2 males that fathered more than 1 litter did so in different years when their 1st mate was no longer present. None of the 9 litters with multiple offspring had more than 1 father. Home-range overlap was mostly between a single male and a single female and with their offspring. All pairs producing offspring were genetically monogamous. Our results strongly support the hypotheses that *M. kikuchii* is socially and genetically monogamous. http://zoolstud.sinica.edu.tw/Journals/51.2/204.pdf

Key words: Genetic monogamy, Home range, Parentage analysis, Social monogamy.

Mating systems of mammals can be defined as monogamous, polygynous, polyandrous, or promiscuous based on the number of partners each individual has (Wittenberger 1979, Clutton-Brock 1989). In the past, a species' mating system was indirectly determined by sexual dimorphism, space use, and mating behaviors (Emlen and Oring 1977, Getz and Hofmann 1986, Carter et al. 1995). Monogamy occurs in < 3% of mammal species (Kleiman 1977) and has attracted much research interest. Traditional ways to determine monogamy include 1) pair bonds between males and females in reproductive and non-reproductive seasons (Carter et al. 1995), 2) aggressive behaviors toward strange individuals (Carter et al. 1995, Back et al. 2002), 3) bi-parental care (Solomon 1993a, Patris and Baudoin 2000), 4) regulation of social factors (e.g., estrus induction) (Carter et al. 1995), 5) the same home range sizes for males and females (Gaulin and FitzGerald 1988), and 6) shared use of a territory (e.g., strong overlap in home ranges) (Reichard 2003).

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These traditional methods provide clues for social monogamy, but not for genetic monogamy. Social monogamy indicates that 1 male and 1 female show social living behavior, but it infers no sexual or reproductive patterns (Reichard 2003). Genetic monogamy is when 1 male and 1 female have an exclusive reproductive relationship, and there are no extra-pair copulations (Reichard 2003).

With the development of molecular genetic techniques, genetic data are being used to examine mating systems (Queller et al. 1993, Avise 1994). Social mating systems may differ from genetic mating systems (Clutton-Brock and Isvaran 2006). In small mammals, for example, *Neotoma cinerea* in North America was thought to be socially polygynous based on sexual dimorphism and female clustering (Finley 1958, Escherich 1981). It was identified as genetically monogamous using DNA fingerprinting techniques (Topping and Millar 1998). In contrast, socially monogamous species were found to engage in extra-pair copulations suggesting they are not genetically monogamous. These include the genetically promiscuous *Apodemus sylvaticus* in the UK (Baker et al. 1999) and the genetically polygynous *A. argenteus* in Japan (Ohnishi et al. 2000). *Peromyscus polionotus* (Foltz 1981) and *P. californicus* (Ribble 1991); however, are both socially and genetically monogamous. To distinguish between social and genetic components of a mating system (Hughes 1998), monogamous mating systems should be examined with field observations and genetic analyses that indicate parentage of offspring and rule out extra-pair copulations (Kraaijeveld-Smit 2004).

In *Microtus* species occurring in the New and Old Worlds (Hoffmann and Koeppl 1985), promiscuousness and polygyny are common, but monogamy is rare (Wolff 1985). *Microtus kikuchii* is the only *Microtus* species endemic to Taiwan. It is the southernmost Old World *Microtus* species (Hoffmann and Koeppl 1985). It lives in diverse habitats, such as grasslands, scrub, and forests, including coniferous, broadleaf, and mixed coniferous and broadleaf forests. It is mainly found at elevations of > 2000 m in alpine habitats of coniferous forests and Yushan cane (*Yushania niitakayamensis*) grasslands. The reproductive season is from Mar. to Aug. (Lu 1991). Chen et al. (2006) studied the behavior of *M. kikuchii* in captivity, and found that when given the freedom to choose, it spent significantly more time with its mated partner than with strange individuals. They also observed paternal care of offspring. Wu (1998) studied the home ranges of *M. kikuchii* using radio-tracking and field trapping. He found that home range sizes did not differ between males and females, only opposite sexes had overlapping ranges, and each range overlapped with only 1 individual of the opposite sex. Those results suggest social monogamy. To date; however, there has been no study of the genetic components of *M. kikuchii* mating systems.

Parental care by both parents and pairing exclusivity are supporting behavioral components of monogamy (Solomon 1993b, Carter et al. 1995, Patris and Baudoin 2000). These home-range and behavioral observational studies led us to hypothesize that *M. kikuchii* is socially and genetically monogamous. Since microsatellite DNA can be sensitive enough to identify parental relationships, relatedness, and dispersal rates (Scribner and Pearce 2000), we used microsatellite DNA and capture-recapture methods to identify consistencies between the social and genetic mating systems of *M. kikuchii*. We tested the following predictions: 1) adult home-range sizes do not significantly differ between sexes, 2) home-range overlaps among adults during reproductive seasons are extensive or exclusive to a single individual of the opposite sex, and 3) there is a lack of evidence of males mating with more than 1 female at the same time (both females alive) or of litters sired by multiple males.

MATERIALS AND METHODS

Trapping

Trapping was carried out from June 2004 to Sept. 2005 in the Hehuan Mt. area (121°17'17.4"E, 24°08'36.4"N) of Taroko National Park, central Taiwan. The elevation is about 3000 m. The dominant vegetation is Yushan cane grassland. Sherman traps baited with sweet potato were set up in a 4-ha (200 × 200-m) grid. To reduce trapping mortality and help retain warmth in cold months, balled-up wads of shredded paper were put in front of the trigger of each Sherman trap. Previous trapping results with 10-m trap spacing in the same Hehuan Mt. area revealed mean home range sizes of 447.9 m^2 in spring, 423.4 m^2 in summer, 258.3 m^2 in fall, and 210.6 m^2 in winter with no statistical differences among seasons or between sexes (Wu 1998). Radio-tracking of 8 individuals for at least 24 h of continuous tracking revealed a mean home range size of 843 m^2

(202-2260 m^2) and a > 20-m movement distance between radio locations in a single day (average 47.5 m, range 22-89 m) (Wu 1998). To maximize the number of individuals trapped, we chose a 20-m trap spacing to form a 40,000-m^2 grid with 121 Sherman traps. In an attempt to catch all members of each family group, we trapped 5 successive nights each month using the capture-recapture method. Because activity of *M. kikuchii* peaks at 04:00-10:00 and 16:00-21:00 (Wu 1998), we checked traps 3 times a day at 05:00-07:00, 09:00-11:00, and 19:00-22:00.

We used toe-clipping to mark each vole the 1st time it was caught. Clipped toes and additionally clipped pieces of the left ear and tail were preserved in 99.9% alcohol for the genetic analysis. For each capture, we recorded the trap site, individual number, sex, age, body weight, and reproductive status (e.g., whether the female nipple size indicated it was pregnant or lactating and whether a male had descended testes). Adults were distinguished from immature voles by the reproductive condition or weight (adult > 30 g).

Home-range size and overlap

Adult individuals captured more than 10 times (Swilling and Wooten 2002) and residing over a month within the trapping grid (i.e., trapped in at least 2 different but not necessarily consecutive monthly trap sessions) were considered residents and used for the home-range analysis. Although Seaman et al. (1999) suggested ≥ 30 relocations for a home range to reduce bias, it was almost impossible to achieve this number of trapping locations because of our regime of 4 trap nights per month and the short life of voles. Therefore, we calculated home ranges for individuals captured in at least 2 monthly trap sessions and with ≥ 10 locations. Individuals caught in only 1 monthly trapping session were not included in the home-range analysis. Home-range sizes were estimated for the reproductive and non-reproductive seasons. ArcView GIS 3.3 (ESRI 1996) with animal movement analysis (Hooge and Eichenlaub 2000) was used to estimate the minimum convex polygon (MCP) home-range size (m^2) from trapping locations. The percentage of home-range overlap between males and females was subsequently calculated. Because of the small sample sizes and dependency of some individuals on home ranges in both the reproductive and non-reproductive seasons, the nonparametric Mann-Whitney *U*-test was used to compare home-range sizes between adult males and females and between reproductive and non-reproductive seasons. Home-range overlap was examined for each 3-mo period in the reproductive seasons to ensure that home ranges overlapped at least part of the time. For example, examination of home-range overlap from Mar. to May could guarantee temporal overlap because individuals in the home-range analysis were captured in at least one of the following scenarios: Mar.-Apr., Apr.-May, or Mar.-May. Thus, ranges of individuals trapped in two of these 3 sessions would overlap temporally. The percentage of home-range overlap was only calculated between resident females and males.

Selection of microsatellite primers

There are no primers designed for *Microtus kikuchii*. *Microtus montebelli* and *M. oeconomus* are the most closely related species to *M. kikuchii* (Conroy and Cook 2000). Therefore, we tested primers for loci MSMM-1-8 designed for *M. montebelli* (Ishibashi et al. 1999) and for loci Moe1-8 designed for *M. oeconomus* (Van de Zande et al. 2000) to determine their suitability for analyzing *M. kikuchii* microsatellite DNA sequences.

Genomic DNA was extracted from left-ear tissue with a DNA purification kit (Epicentre, Madison, WI, USA). The above primers amplified specific sequences. A polymerase chain reaction (PCR) used a total volume of 50 μl with 1 μl of a fluorescence-labeled forward primer (25 mM), 1 μl of an unlabeled reverse primer (25 mM), 5 μl PCR buffer (10x), 0.6 μl DNA, 0.6 μl DNTP (10 mM), 0.6 μl *Taq*, and 41.2 μl water. Because the lengths of these PCR products were too short for direct sequencing, they were excised from the agarose gel for TA cloning. Each specific sequence was ligated with a vector (Invitrogen, Grand I., NY, USA) and put into competent cells for TA cloning. All clones were further re-amplified with M13 primers (forward and reverse) which were supplied with TA cloning kit (Invitrogen) for length check. PCR protocol of the TA cloning check started from denaturation at 94°C for 10 min. Twenty-five cycles were performed at the following conditions: 1 min at 94°C, 1 min at 55°C for annealing, and 1 min at 72°C for extension. Horizontal electrophoresis with a 2% agarose gel was used to check the sequence lengths of the clones.

Clones containing sequences of < 500 bp (Schlotterer and Harr 2001) were sent to Mission Biotech Company (Taichung, Taiwan) to be sequenced on an ABI PRISM™ 3730*xl* DNA

Analyzer (Applied Biosystems, Carlsbad, CA, USA). Usable loci were determined using BioEdit 6.0.5 (Hall 1999) to check each sequence for repeating units and whether both sides of the sequence were conserved and stable. Primers of usable microsatellite loci were used to synthesize fluorescent primers for the microsatellite analysis.

Genetic data analysis

For the microsatellite analysis, protocols for DNA extraction and the PCR were the same as those described above. PCR products were separated on an ABI 310 genetic analyzer (Applied Biosystems). Individuals were genotyped using Genotyper vers. 2.0 (Applied Biosystems).

Tests of pairwise linkage disequilibrium between loci were conducted using GenePop (Raymond and Rousset 1995). Allele diversity, heterozygosity (observed *Ho* and expected *He*), and Hardy-Weinberg equilibrium of each loci were calculated and tested using GENALEX 6 (Peakall and Smouse 2006).

CERVUS 2.0 (Slate et al. 2000) and GENALEX 6 (Peakall and Smouse 2006) were used to estimate parentage. Since the real parentage of any individual could not be assured based on the capture data, we randomly compared genotypes of all individuals to all males to identify the most likely fathers. These offspring-father pairs were randomly compared to all females to identify the most likely mothers. An error rate of 1% was incorporated into the simulation with 80% relaxed and 95% strict confidence intervals. Parentage was confirmed on the basis of mismatching putative parentage at 0 loci or 1 locus, the LOD score (log-likelihood of each candidate parent), and the confidence of ΔLOD (the difference between the 2 most likely parents). A ΔLOD score of > 3.0 confirmed parentage, while a ΔLOD score of < -3.0 rejected parentage (Slate et al. 2000). A ΔLOD score was calculated by the difference in LOD scores between the most likely and the 2nd most likely candidate parents (either of which might be the true parent). The most likely candidate parent was the one with a ΔLOD score exceeding the critical ΔLOD score with a 95% confidence interval. Relationships of individuals with overlapping home ranges were checked with results of the parentage analysis to see whether they were mates or family members.

RESULTS

In total, 169 voles (79 males and 90 females) were caught in 1615 captures. One vole was excluded from the parentage analysis due to failure to amplify its DNA.

Polymorphism of microsatellite loci

In total, 11 microsatellite loci were chosen. Seven loci (MSMM-2, -3, -4, -5, -6, -7, and -8) used primers designed from *Microtus montebelli* and 4 loci (Moe1, -2, -5, and -6) used primers designed from *M. oeconomus* (Table 1). Except for MSMS-7, numbers of alleles were > 10; averaging 14.3 (range 8-19). All amplified loci were polymorphic. The observed heterozygosity (*Ho*) value of each locus was close to the expected heterozygosity (*He*) value. Average values of *Ho* and *He* were both 0.88. As a result, the mean inbreeding coefficient, *F*, was essentially 0 at -0.002. There were no departures from Hardy-Weinberg equilibrium (Table 1), indicating that the study population was under Hardy-Weinberg equilibrium. Locus pairs MSMM-4/MSMM-7 and MSMM-4/Moe5 showed significant linkage disequilibrium. Most loci showed no significant linkage disequilibrium. Therefore, locus MSMM-4 was not used for the genetic analysis.

Parentage analysis

The critical ΔLOD with a 95% level of certainty was 0.08 (with 95% of the parentage resolved) if neither parent was known. Of the total 168 voles used for the parentage analysis, 20 mated pairs were found (18 males and 20 females) to have 31 offspring. In total, 69 voles were assigned parentage (Table 2). In other words, 41.1% (69/168) of the 168 voles, including adult and immature voles, could be assigned parentage with both parents identified. Except for 2 males (49M and 50M), each male mated with only 1 female during the study period. The 2 males who mated with more than 1 female did so in different breeding seasons in different years and after the 1st female was no longer present.

Home-range size and overlap and their relationships

The home-range sizes were 2763.6 ± 2228.5 m^2 (n = 22) for adult males and 2170.0 ± 1341.3 m^2 (n = 20) for adult females. No significant

difference was found between sexes (Mann-Whitney *U*-test, $p = 0.31$). Average home-range sizes in the reproductive season were 1826.1 ± 1463.9 m^2 ($n = 23$) for adult males and 1684.2 ± 1256.7 m^2 ($n = 19$) for adult females. The average home-range sizes in the non-reproductive season were 2072.7 ± 1637.7 m^2 ($n = 11$) for adult males and 1125.0 ± 874.6 m^2 ($n = 8$) for adult females. No significant difference in home-range sizes was found between sexes (Mann-Whitney *U*-test, $p = 0.74$ for the reproductive season and $p = 0.16$ for the non-reproductive season). The sample size for the non-reproductive season was small.

We separated the reproductive season into 3 periods of 3 mo each and compared the home-range overlap among resident individuals (Fig. 1). Only those with ranges that overlapped ranges of the opposite sex are shown. There were 21 pairwise combinations of home ranges overlapping those of the opposite sex. Eleven (52.4%) showed exclusive home-range overlap between 1 male and 1 female (Fig. 1). Eight (38.1%) of these 21 pairwise combinations between male and female ranges were detected as sexually paired partners. Six (75%) of these 8 reproductive pairs had exclusive, overlapping home ranges. The average overlap of a male's range with a female's range did not statistically differ from the average overlap of a female's range by a male's range (Wilcoxon rank-sum test, $p = 0.126$). Average percentages of a female's home range overlapped by a male's were significantly larger in mated pairs (77.1%, $n = 8$) than in pairs not found to produce offspring (41.8%, $n = 11$) (Mann-Whitney *U*-test, $p = 0.0372$). Average percentages of a female's home range overlapped by a male's were also significantly larger than a male's home range overlapped by his sexual female partner's (42.4%, Wilcoxon rank-sum test, $p = 0.0499$, $n = 8$). In other words, a female's home range tended to overlap more with that of her sexual partner, but males did not show this trend.

In total, 14 social units were recognized based on overlapping home ranges involving

Table 1. Microsatellites used in the study of *Microtus kikuchii* at Hehuan Mt., Taiwan, from June 2004 to Sept. 2005. Microsatellite variations include the annealing temperature (T*a*), number of alleles, observed heterozygosity (*Ho*), expected heterozygosity (*He*), inbreeding coefficient (*F*), Hardy-Weinberg equilibrium (H-W), and *p* value (*p*)

Locus	Core sequence	T*a* (°C)	Sequence (5'-3')	Allele size range (bp)	Number of alleles	*Ho*	*He*	*F*	H-W	
										p
MSMM-2[a]	$(CA)_{21}$	52	TAACCACAACCCCTCCAACTG TCATTTGGAGTTGCTGAGAAC	163-197	15	0.893	0.900	0.007	NS	0.627
MSMM-3[a]	$(CA)_{15}$	52	TACGCCCTTCAAACTCATGTG TCCTTTATCTTAGGTGATGGAG	102-136	14	0.833	0.827	-0.008	NS	0.279
MSMM-4[a]	$(CA)_{19}$	52	TGTTTCAAGGCAATAAGGTGG TCGTTTCCCTGGAGATTGGG	143-187	18	0.913	0.872	-0.047	NS	0.512
MSMM-5[a]	$(CA)_{17}$	52	TCTAATACCCTCTTCCTTGGG TCCTATCAAGGGGCATTCATCT	69-111	19	0.900	0.910	0.011	NS	0.247
MSMM-6[a]	$(CA)_{20}$	52	TCCTATCAAGGGGCATTCATCT TACAAAGCCATTGTTCCCTGCT	145-167	12	0.873	0.857	-0.019	NS	0.987
MSMM-7[a]	$(CA)_{18}$	56	TAAGAAGGGCCACTAAGACCC TGGGATTAAAGGTGTGCACCA	105-123	8	0.820	0.830	0.012	NS	0.915
MSMM-8[a]	$(CA)_{17}$	50	TGCTTAGTTCACTGCTGAACC TCTTACTATCTGTCATTGAAGA	170-196	13	0.927	0.886	-0.046	NS	0.246
Moe1[b]	$(GT)_{18}$	60	TGGTTGTTCTGTGGTGAATACAG ACAGTAAGCAGTTTATCCACAAACC	93-133	15	0.847	0.890	0.049	NS	0.770
Moe2[b]	$(GT)_{17}$	60	CATCTGATGAGTCCCTGAGG GCAACCTTCTTCTGACTTTTAC	145-185	15	0.887	0.889	0.002	NS	0.480
Moe5[b]	$(TC)_{25}$	60	GGTCATGCTCCAAGAAGCTC AAAACCAAGGGTGCTGCTC	108-138	14	0.833	0.866	0.037	NS	0.663
Moe6[b]	$(GT)_{25}$	60	GGTTTTCTGATTCAGGCAGG CCTCTTCTGGCCTCTCCAG	210-246	14	0.913	0.998	-0.017	NS	0.420

[a]Ishibashi et al. (1999). [b]Van de Zande et al. (2000). NS, non-significant.

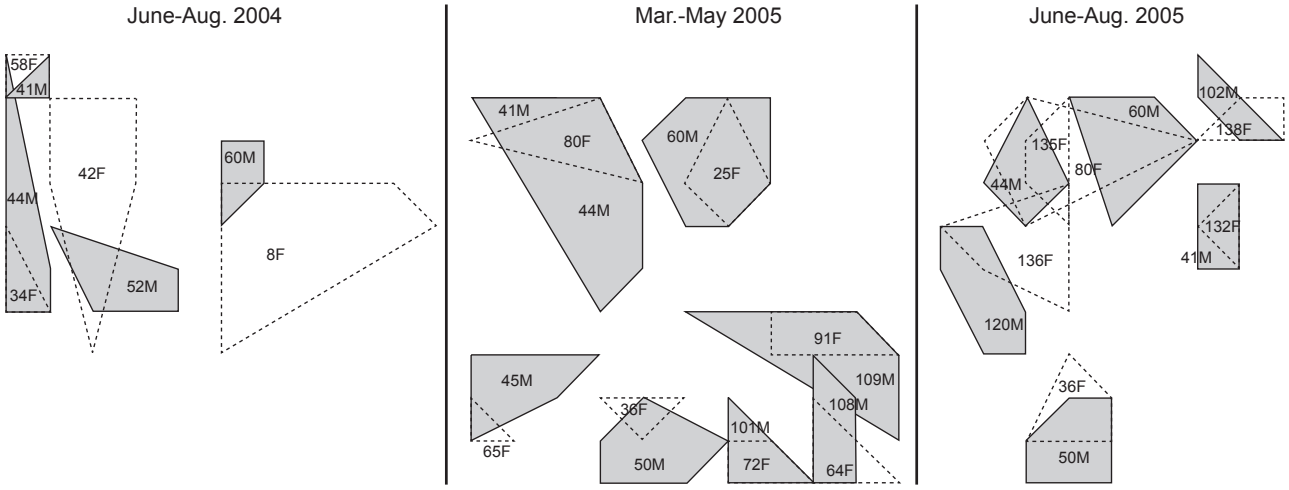

Fig. 1. Overlapping home ranges of adult *Microtus kikuchii* at Hehuan Mt., Taiwan, during 3 consecutive reproductive periods. Numbers indicate different individuals. Letters indicate the sex (M for males and F for females). Male home ranges are illustrated with solid-line boundaries and lightly shaded interiors. Female home ranges have bold dotted boundaries. Only overlapping home ranges between opposite sexes are shown.

Table 2. Parentage analysis of *M. kikuchii* at Hehuan Mt., Taiwan

Offspring[a]	Date offspring captured	Parents[a]	Number of mismatched loci between offspring and parents (female/male)	LOD[b]	ΔLOD[c]*
15M	2004 June	2F and 16M	1 / 1	8.06	4.84
4M	2004 June	17F and 13M	0 / 0	8.36	0.55
83F	2004 Sept.		0 / 0	9.64	7.79
5M	2004 June	10F and 50M	0 / 0	11.40	6.31
37M	2004 July	12F and 22M	0 / 0	9.66	7.26
42F	2004 July	24F and 35M	0 / 0	9.59	2.17
46M	2004 July		0 / 0	8.89	3.15
58F	2004 July	19F and 30M	0 / 0	8.03	3.03
43F	2004 July	48F and 49M	0 / 0	9.96	6.77
77M	2004 Sept.	65F and 54M	0 / 0	8.93	4.98
140F	2005 June		1 / 1	8.69	6.40
144M	2005 June		0 / 0	6.14	1.10
82F	2004 Sept.	74F and 90M	1 / 0	8.05	4.74
110F	2005 Mar.		0 / 0	7.39	1.64
70F	2004 Aug.	93F and 68M	1 / 0	9.07	0.46
81F	2004 Sept.		0 / 0	9.65	2.30
91F	2004 Oct.		0 / 0	10.20	2.91
102M	2005 Jan.	18F and 40M	0 / 0	6.84	4.62
111F	2005 Mar.		0 / 0	11.70	10.40
135F	2005 May	80F and 44M	0 / 0	12.80	9.21
136F	2005 May		0 / 0	8.86	4.20
146M	2005 June	91F and 109M	0 / 0	10.10	2.67
157M	2005 July		0 / 0	11.60	3.03
148F	2005 June	58F and 49M	1 / 1	8.45	6.70
150M	2005 June	72F and 101M	1 / 1	8.46	3.80
160F	2005 July		1 / 0	8.03	1.90
153M	2005 July	36F and 50M	0 / 0	9.96	5.52
158F	2005 July	25F and 60M	0 / 0	9.87	7.89
156F	2005 July	152F and 100M	0 / 0	10.50	5.17
164M	2005 Aug.	111F and 102M	0 / 0	6.47	1.08
167F	2005 Aug.	142F and 210M	0 / 0	10.50	8.23

[a]Sex indicated by M (male) and F (female). [b]LOD score, log of product of likelihood ratios at each locus. [c]ΔLOD, difference in LOD score between the most likely candidate parent and the 2nd most likely candidate parent. *All ΔLOD values were significant ($p < 0.05$).

both sexes (Fig. 1). Eleven (78.6%) had overlaps exclusively between 1 male and 1 female. Six of these 11 social units (54.5%) were paired sexually and monogamously produced offspring, and 5 social units were not detected to have produced any offspring. For the 3 social units with > 2 individuals, only the social unit with 2 males and 2 females (June-Aug. 2004) was not detected to have reproduced. The 2nd social unit (Mar.-May 2005) had a mated pair (44M-80F). The 3rd (June-Aug. 2005) had a family group (parents 44M-80F and 2 daughters: 135F and 136F). No sexual pairing or family groups were found in June-Aug. 2004. In 2005, all social units found to reproduce (8 of 10, 80%) practiced monogamy and were either mated pairs or family groups. In total, 8 (57.1%) social units were found to have produced offspring. Six (75%) of these had exclusive home-range overlap between 1 male and 1 female.

DISCUSSION

Our results strongly support the hypotheses that *Microtus kikuchii* is socially and genetically monogamous. We identified litters sired by 18 males and 20 females. The 2 males that fathered more than 1 litter did so in different years and with a different mate because their 1st mate was no longer present. None of the 9 litters with multiple offspring had more than 1 father (Table 2). We found no significant differences in home-range sizes between sexes. Most (78.6%) social units consisted of only 1 male and 1 female. Home-range overlaps between male and female pairs not found to produce offspring were relatively small (44M-58F, 41M-80F, and 120M-136F), except for 60M and 80F. Therefore, overlap in home range was mostly by a single male, a single female, and their offspring, strongly suggesting social monogamy.

Microtus kikuchii was suspected of being socially monogamous based on home ranges (Wu 1998) and observations of captive individuals (Chen et al. 2006). Combining our home-range data with a genetic analysis of parentage provided more in-depth information on the relationships of voles observed to have overlapping home ranges. *Microtus ochrogaster* is considered a socially monogamous species (Hofmann et al. 1984, Carter et al. 1995) even though not all adults live as male-female pairs. Adults live in groups of single males and females, and groups of 3 or more adults were also documented (Getz et al. 1993, Cochran and Solomon 2000, Lucia et al. 2008). We maintain that *M. kikuchii* is socially monogamous because of similar home-range sizes between sexes, the very high proportion of social units comprised of male-female pairs, and the relatively low overlap in home ranges of individuals without reproductive relationships (e.g., not sexual partners or family members).

Only six of the 11 male-female pairs identified by overlapping home ranges were found to be paired partners that had successfully produced young. Male-female pairs determined by overlapping home ranges might not truly be breeding pairs. In *M. ochrogaster*, socially monogamous, multiple paternity in five of 9 litters was also identified by a genetic study (Solomon et al. 2004). Thus, data from home-range overlap cannot reflect true pairing conditions or whether *M. kikuchii* is genetically monogamous. To determine the mating system of a species, field data and genetic data are both necessary (Hughes 1998, Kraaijeveld-Smit 2004). As we found no litters sired by multiple fathers, genetic monogamy of *M. kikuchii* should be assured. The parentage analysis showed survival of 1 or 2 offspring in each litter. This is consistent with Lu's (1991) data from field trapping (range 1-3, average litter size 2.1) and our own observations from captive breeding (litter size 1-3 with 2 most frequent) (pers. unpubl. data). With an average litter size of 2.1, it may be more difficult to detect multiple paternity in *M. kikuchii* than for species with larger litter sizes, such as *M. ochrogaster*. Low detectability of multiple paternity due to small litter size is unlikely for *M. kikuchii* because Lu (1991) found a very low post-implantation mortality rate (2 resorbed embryos of 64 embryos). Moreover, we are confident that we trapped most of the population because our extensive trapping effort spanned 2 breeding seasons, and we had high recapture rates (71.5%-96.5%). Even so, we still found no litters sired by multiple fathers.

Some studies are beginning to show that in a number of species considered to be monogamous, females mate with multiple males. In mammals, extra-pair copulation was found in some socially monogamous species (Richardson 1987, Agren et al. 1989, Solomon et al. 2004, Mabry et al. 2011). Previously, only 2 known rodent species simultaneously showed social and genetic monogamy, i.e., *Peromyscus polionotus* (Foltz 1981) and *P. californicus* (Ribble 1991). Genetic promiscuity and polygyny are common in *Microtus* (e.g., *M. pennsylvanicus*, *M. richardsoni*,

M. xanthognathus, and *M. californicus*), but monogamy is rare (Wolff 1985). Our study has added *M. kikuchii* to the list of rodent species simultaneously showing social and genetic monogamy.

Acknowledgments: The authors thank the anonymous reviewers for their critical comments and many valuable remarks on the original manuscript. We thank Q.W. Zhu, J.K. Lin, G.H. Zhen, S.L. Yuan, and many other volunteers for help with fieldwork. We thank M.Y. Zhen, S.F. Zhen, Z.X. Zhang, L.Y. Liu, R.P. Huang, W.Y. Zhen, and Z.Y. Guo for help with genetic work. We thank the high-elevation experimental station of the Taiwan Endemic Species Research Institute for accommodations during fieldwork. Trapping protocols complied with government regulations. Permits were obtained from Taroko National Park. This research was funded by the National Science Council of Taiwan (NSC96-2621-B-029-001-MY3) and Tunghai Univ.

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