Journal of Mammalogy, 86(3):531–540, 2005

PARENTAGE ANALYSIS OF THE CANYON MOUSE (*PEROMYSCUS CRINITUS*): EVIDENCE FOR MULTIPLE PATERNITY

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Mating systems within the genus *Peromyscus* have traditionally been inferred from field observations of spatial relationships of males and females and from laboratory behavioral studies. The majority of species within *Peromyscus* are assumed to be promiscuous, but rarely have these conclusions been verified with molecular data. Genotypes constructed from 4 microsatellite loci were used to determine paternity in 10 litters of the canyon mouse (*Peromyscus crinitus*). We applied 3 criteria for paternity assignment: direct observation of paternal alleles, correlation of sampled male and offspring genotypes (mothers known) including spatial relationship data, and application of a likelihood estimator. Multiple paternity occurred in a minimum of 20% of the litters, yet we found no evidence that would indicate that any putative father sired 2 or more litters. Dispersal of males between the time of copulation and parturition was minimal (7.5–70 m) in most cases, but 1 male was captured in a rock outcropping separate from that of his sired litter (174 m). Our use of 3 different criteria allowed a greater number of male paternity assignments to be inferred than would have been possible when using software program CERVUS alone, and increased confidence in these results.

Key words: mating system, microsatellites, multiple paternity, parentage analysis, *Peromyscus crinitus*

Understanding mating systems, especially those characterized by multiple and extrapair paternity, is important because of the fundamental role that multiple paternity plays in the evolution of many life-history strategies (Griffeth et al. 2002). Multiple paternity not only increases effective population size (relative to single paternity—Sugg and Chesser 1994) and affects genetic variability and evolutionary potential (Valenzuela 2000), but it also can be an indicator of the intensity of sexual selection and sperm competition (Fitzsimmons 1998; Kelly et al. 1999; Reynolds 1996). A better understanding of mating behavior and breeding outcome can allow for development of a more unified body of theory that describes the relationship between ecological factors and mating patterns (Conrad et al. 2001).

Mating systems are a result of the reproductive behaviors of individuals (Gomendio et al. 1998)—the basic unit upon which natural selection operates within populations (Emlen and Oring 1977). Price and Evans (1991) described 2 distinct aspects of a species' reproductive strategy: mating system (i.e., who copulates with whom), and breeding system (which we term "genetic breeding system" herein to distinguish from the breeding system as defined by Reynolds [1996]). This refers to who actually contributes genes to the next generation. Thus, the genetic breeding system is only one part of the holistic idea of a mating system (Emlen and Oring 1977).

Deer mice (genus *Peromyscus*) are ubiquitous across North America and likely are the most abundant native mammal group on the continent. Because of their accessibility and impressive adaptive radiation (about 53 species—see Musser and Carleton 1993), deer mice represent a unique evolutionary model and have been referred to as "the *Drosophila* of North American mammalogy" (Dewey and Dawson 2001; Musser and Carleton 1993). Nevertheless, relatively little is known about social organization and mating systems of *Peromyscus* in nature because of their nocturnal habits and small size (Dewsbury 1981). As in many other taxa, mating systems within *Peromyscus* have been inferred mostly from field studies

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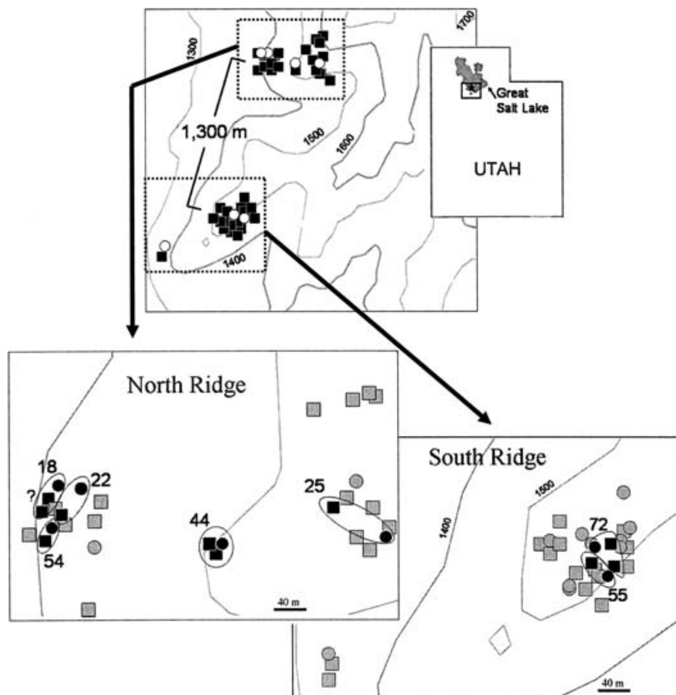

Fig. 1.—Study site for *Peromyscus crinitus* on Stansbury Island, Tooele County, Utah. Circles represent females and squares represent males. In upper diagram, black squares represent males and white circles represent females. Lower diagrams of the north and south ridges are more detailed and are keyed as follows: black circles = pregnant females, gray circles = nonpregnant females, black squares = males assigned to a litter, and gray squares = males not assigned to a litter. Breeding pairs or groups are circled. Numbers correspond to pregnant females and are consistent with QRS specimen numbers throughout the paper. Question mark indicates a breeding pair for which the father cannot be assigned between 2 adult males. (Map graphics courtesy of Garmin Ltd., Romsey, United Kingdom)

of spatial relationships of males and females and from laboratory behavioral studies (Wolff 1989). The application of molecular methods has proven most useful in situations where specific testable hypotheses about the social mating system of the species in question have been developed. Dewsbury (1981) associated several behavioral and physiological correlates with monogamy in rodents. Based on these correlates, he accurately predicted that *P. californicus* (Ribble 1991) and *P. polionotus* (Foltz 1981) should be monogamous and that the promiscuous species *P. maniculatus* (Birdsall and Nash 1973; Ribble and Millar 1996) and *P. leucopus* (Xia and Millar 1991) were not likely to be monogamous.

The canyon mouse (*Peromyscus crinitus*) is endemic to western North America and usually is associated with sheer cliffs and slickrock (i.e., smooth, wind-polished rock). Being one of the relatively less-known *Peromyscus* species, the literature is devoid of conclusive evidence as to the mating or genetic breeding system that characterizes *P. crinitus*. Dewsbury (1981) underscored this point when he acknowledged that he could only use one-half of the proposed correlates for *P. crinitus* to infer a system of mating because of a lack of data. Even so, Dewsbury (1981) predicted that the canyon mouse is not likely to exhibit a monogamous mating system (although he acknowledged that it is risky to make predictions based on only a few correlates).

We used microsatellite DNA markers to determine patterns of paternity in the canyon mouse, thereby testing the hypothesis that *P. crinitus* is characterized by a mating system in which polygamy occurs, as suggested by Dewsbury (1981). In addition to analyzing genotypic data from known mothers and putative fathers, we also incorporated linear distances between males and pregnant females as an additional character to facilitate differentiation among similar male genotypes, to investigate movements of males within the first 2–3 weeks after conception, and to better characterize the mating system of *P. crinitus*. We predicted that because of the patchy nature of the habitat in which *P. crinitus* is found, successfully breeding males would be located in the same rock outcropping as their breeding partner.

Materials and Methods

Study Site and Sampling

All *P. crinitus* examined, with the exception of 3 pregnant females, were collected between 10 May and 1 June 2002 on Stansbury Island, a peninsula that extends north into the Great Salt Lake, Tooele County, Utah (approximately 60 km west of Salt Lake City, Salt Lake County, Utah), hereafter referred to as "Stansbury Island" (Fig. 1). The study site consists of granite peaks that rise more than 700 m above the elevation of the lake. Specimens were collected from granite outcroppings and slickrock on 2 rocky ridges that were separated by approximately 1 linear kilometer. The upper one-third of the southernmost ridge was composed of continuous rock, but the northern ridge consisted of several rocky "islands" surrounded by cheat grass (*Bromus tectorum*), which comprised the majority of the vegetation. A dry canyon devoid of major rock outcroppings separated the northern from the southern ridges. *P. crinitus* sample size was augmented by the addition of 3 pregnant females from Uintah and Kane Counties, Utah (280 and 400 km from Stansbury Island, respectively; see Appendix I), to increase the number of litters examined. Specimens DSR4423 and DSR4436 (Uintah County) were collected in May 1995 and DSR5650 (Kane County) was collected in August 1999. These 2 sites are separated by 360 km. Specimens are given initials of collectors (QSR, the 1st author, and DSR, the 3rd author).

Rock outcroppings were sampled by using Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) and snap traps (baited with birdseed, moistened oats, or peanut butter) set approximately 5 m apart. All captured *P. crinitus* were processed as voucher specimens (skin plus skeleton) and archived in the Monte L. Bean Life Science Museum mammal research collection at Brigham Young University. Tissue samples and embryos were stored in the field on dry ice and later in the laboratory at −20°C. Special care was taken to prevent contamination of embryos with littermates or maternal tissue (see Shurtliff 2003). Locality data were recorded by using a Garmin eTrex Legend global positioning system unit (Garmin Ltd., Romsey, United Kingdom) at each capture site. Additionally, distances were measured manually between all capture sites that fell within 40 m of each other. This was necessary because of the error associated with the global positioning system unit, which only afforded maximum accuracy to within ~5 m. Collections were made under appropriate permits from the state of Utah, and methods of animal handling and euthanasia conformed to guidelines of the American Society of Mammalogists for

Table 1.—Summary statistics from loci used for study of *Peromyscus crinitus* in Utah, including observed (H_O) and expected (H_E) heterozygosity and Hardy–Weinburg equilibrium (H–W). Abbreviations: bp = base pairs, NS = difference between observed and expected heterozygosity not significant. Null allele frequency was estimated by software program CERVUS and is an estimate of the proportion of alleles that do not amplify because of a mutation in the flanking region.

Locus	Core repeats	Annealing temp (°C)	No. alleles	Fluorescent markers	Allele size range (bp)	H_O	H_E	H–W	Null allele frequency
Pml03	$(CA)_{22}$	50	10	6-FAM	232–262	0.810	0.838	NS	0.0168
Pml08	$(CA)_{20}$	50	9	NED	204–250	0.492	0.669	NS	0.1515
Pml09	$(CA)_{25}$	50	8	NED	191–247	0.698	0.758	NS	0.0398
PLGT62	$(GT)_{20}$	50	9	HEX	151–203	0.694	0.752	NS	0.0382

the capture, handling, and care of mammals (Animal Care and Use Committee 1998).

Microsatellite Analysis

Total genomic DNA was extracted from kidney, liver, heart, or spleen tissue of adult animals following, generally, the procedure of Fetzner (1999). Exceptions to this protocol are described in Shurtliff (2003). Because of limited amounts of embryonic tissue, we isolated genomic DNA from embryos by using a DNeasy Tissue Kit (Qiagen Inc., Valencia, California). By screening 27 microsatellite loci reported by Schmidt (1999) and Chirhart et al. (2000), we were able to identify 4 loci that were polymorphic in samples of *P. crinitus* (Table 1).

Amplifications were performed in an ABI GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, California) and were initially denatured at 94°C for 2 min, after which 30 cycles of the following steps were performed: denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and polymerization at 72°C for 45 s. Resulting polymerase chain reaction products were visualized on agarose gels with a GIBCO 100–base-pair DNA ladder (BRL#15628-019, Invitrogen Co., Carlsbad, California) as a standard.

Allele sizes were resolved on a 3100 Genetic Analyzer (Applied Biosystems) at the Brigham Young University DNA Sequencing Center and products of each locus were scored by using GENOTYPER (Perkin-Elmer Corp., Foster City, California). Maternal and embryonic samples with homozygous alleles were processed again through polymerase chain reaction and genotyped a 2nd time to ensure that all homozygote loci yielded consistent results. Additionally, any locus that produced a weak signal relative to others in the same processing was reamplified and genotyped.

Data Analysis

We used several indicators to measure the ability of our markers to distinguish between individual genotypes. The paternal exclusion probability (Jamieson 1965; Selvin 1980), which is the probability of excluding a male that is not the true father, was calculated across 4 loci given that 1 parent was known. The mean probability of identity values across all loci was calculated as in Paetkau and Strobeck (1994). A value for probability of identity is the probability that 2 animals chosen at random from the same population would have identical genotypes. These confidence values can be biased if breeding animals within the study population are close genetic relatives (Waits et al. 2001). Therefore, we also calculated the probability of identity for full siblings (Evett and Weir 1998; Waits et al. 2001). This statistic gives a conservative lower limit of confidence for multilocus genetic matches if close relatives are present in the sample.

Parentage Analysis

In this study we used 3 approaches to assign paternity in litters of *P. crinitus*.

Criterion 1: exclusion.—The 1st approach was to identify manually paternal alleles in each litter by using methods in Burton (2002), after having accounted for maternal alleles. Occasionally, either allele of a locus could have come from the mother in more than 1 offspring, making it impossible to distinguish whether 1 or 2 different paternal alleles were present (for an example see locus Pml03 for the litter of female QRS18; Table 2). In such cases we took the conservative path relative to our hypothesis and assumed that such represented only 1 paternal allele. However, we did not exclude any male that had either of the alleles in question. In this way, a hypothetical paternal genotype was constructed for each litter. Because of high mutation rates in microsatellites and the possibility of typing errors, we followed a conservative approach and only designated a litter as multiply sired when we detected 3 or more paternal alleles at 2 or more loci (see Burton 2002; Fitzsimmons 1998; Valenzuela 2000). All litters of *P. crinitus* examined in this study were analyzed according to this criterion.

Criterion 2: male genotypes and dispersal distances.—The hypothetical paternal genotype obtained from criterion 1 was correlated with male genotypic and spatial relationship data. All sampled male genotypes were compared manually in a pairwise fashion to each litter from Stansbury Island that had ≤ 2 paternal alleles per locus. A male whose genotype matched the hypothetical paternal genotype obtained from criterion 1 was considered the sire of that litter. If no sampled male genotype matched the hypothetical paternal genotype the locus containing the discrepancy was examined to determine if a single mutation could account for nonassignment. Any discrepancy in more than 1 embryo at a single locus was considered evidence that the actual father was not sampled. Such a litter was designated as "paternity unknown."

We also analyzed litters that had 3 paternal alleles at only 1 locus (and were thus considered singly sired under criterion 1). Theoretically, it is possible that a multiply-sired litter could have no more than 2 paternal alleles at each locus. Hence, we did not automatically exclude a male from being the father of the entire litter in this situation (i.e., with 3 paternal alleles at 1 locus) because a single allele mismatch could have resulted from a typing error, mutation, or null allele (Marshall et al. 1998). If 1 male genotype matched all alleles across all loci within a litter, but failed to match a 3rd allele at a single locus, that mouse was assumed to be the father of the litter and the discrepant 3rd allele was assumed to be due to a mutation or typing error. However, when 2 or more males were required to account for paternal alleles at any locus other than the 3-paternal-allele locus, we concluded that either we did not sample the putative father, or more likely, the litter was multiply sired. After putative fathers were assigned paternity, we calculated linear distances between trap localities of potential fathers and pregnant females.

Criterion 3: CERVUS.—Our 3rd approach was to incorporate genotypic data into the likelihood-based computer program CERVUS 2.0 (Marshall et al. 1998), which calculates log-likelihood ratio scores

Table 2.—Genotype data for 10 litters of *Peromyscus crinitus* and CERVUS analysis of putative fathers. Included are maternal genotypes and a hypothetical paternal genotype, which was obtained by subtracting the maternal allele from each embryo genotype at each locus. Alleles are represented by DNA base pairs and are separated by a comma. Blank indicates missing data. Under CERVUS results, the putative father identification and corresponding log-likelihood ratio (LOD) and delta (Δ) values are listed. A value falls within the specified confidence level if delta scores are higher than the critical values of 1.32 for strict (95%) and 0.58 for relaxed (80%) confidence levels. Single asterisks indicate that a value is within the relaxed confidence level. Two asterisks indicate that a value is within the strict confidence level. Males and females are identified by QRS and DSR specimen numbers.

	Locus				CERVUS results		
Individual (litter number)	Pml03	Pml08	Pml09	PLGT62	Putative father identification	LOD	Δ
Female QRS18 (1)	232, 256	230, 230	219, 219	167, 203			
Embryo A	232, 240	230, 230	216, 219	167, 171	20	3.04	0.101
Embryo B	232, 256	230, 230	219, 219	167, 171	38	2.69	0.420
Embryo C	232, 256	230, 230	219, 219	171, 203	38	2.70	0.420
Hypothetical paternal genotype	240, 232 or 256[a]	230	216, 219	171			
Putative father QRS20	232, 240	230, 230	216, 219	171, 171			
Female QRS22 (2)	246, 252	204, 232	216, 234	163, 171			
Embryo A	240, 246	204, 238	216, 241	171, 203	39	3.51	2.660**
Embryo B	240, 246	204, 232	216, 219	163, 165	39	1.88	0.336
Embryo C	240, 252	232, 238	216, 219	163, 203	39	2.77	0.424
Embryo D	246, 246	232, 238	216, 241	163, 165	39	3.20	1.340**
Embryo E	246, 246	204, 238	234, 241	163, 203	39	2.87	1.390**
Hypothetical paternal genotype	240, 246	204 or 232, 238[a]	219, 241	165, 203			
Putative father QRS39	240, 246	238, 238	219, 241	165, 203			
Female QRS25 (3)	232, 256	230, 230	216, 219	169, 169			
Embryo A	232, 240	230, 230	219, 241	169, 173	53	4.00	2.140**
Embryo B	232, 256	230, 230	216, 241	163, 169	53	2.64	0.021
Embryo C	256, 256	230, 230	195, 216	169, 173	53	4.45	2.930**
Embryo D	256, 256	230, 230	195, 216	169, 173	53	4.45	2.930**
Hypothetical paternal genotype	240, 256	230	195, 241	163, 173			
Putative father QRS53	240, 256	230, 230	195, 241	163, 173			
Female QRS44 (4)	236, 236	204, 230	241, 241	169, 171			
Embryo A	236, 246	204, 232	216, 241	171, 171	45	2.12	0.359
Embryo B	236, 246	230, null	196, 241	163, 171	51	2.48	1.280*
Embryo C	236, 246	230, 232	216, 241	169, 171	45	2.50	0.216
Embryo D	236, 252	204, null	196, 241	163, 169	51	3.65	3.090**
Hypothetical paternal genotype	246, 252	232, null	196, 216	163, 171 or 169[a]			
Putative father QRS51	246, 252	232, null	196, 216	163, 171			
Female QRS54 (5)	246, 246	204, 230	195, 219	163, 203			
Embryo A	240, 246	204, 230	195, 195	171, 203	62	3.16	0.564
Embryo B	246, 248	230, 230	195, 219	163, 171	49	2.76	0.815*
Embryo C	240, 246	204, 230	219, 219	163, 171	20	2.10	<0.001
Embryo D	240, 246	204, 230	195, 195	163, 163	49	3.23	0.483
Embryo E	240, 246	230, 230	195, 219	171, 203	62	2.71	0.073
Hypothetical paternal genotype	240, 248	230 (and 204?)	195, 219	163, 171			
Putative father QRS49	240, 248	230, 230	195, 219	163, 171			
Female QRS55 (6)	246, 246	204, null	219, 219	163, 167			
Embryo A	242, 246	204, 220	219, 219	163, 163	33	4.77	2.350**
Embryo B	242, 246	220, null	219, 219	163, 167	33	3.33	2.120**
Embryo C	242, 246	220, null	219, 241	163, 167	33	3.33	.120**
Hypothetical paternal genotype	242	220	219, 241	163 (and 167?)			
Putative father QRS33	240, 242	220, 230	219, 241	163, 163			
Female QRS72 (7)	248, 248	204, 220	219, 219	171, 203			
Embryo A	248, 250	204, 204	195, 219	171, 171	34	4.51	0.834*
Embryo B	246, 248	204, 204	219, 219	171, 171	35	2.02	0.037
Embryo C	246, 248	204, 220	219, 219	171, 171	35	1.97	0.083
Embryo D	246, 248	204, 204	219, 241	173, 203	37	2.77	1.510**
Hypothetical paternal genotype	246, 250	204 and/or 220	195, 219, 241	171, 173			
Putative father QRS34	246, 250	204, 204	195, 219	171, 171			
Putative father QRS37	246, 250	204, 220	219, 241	163, 173			

Table 2.—Continued.

Individual (litter number)	Locus				CERVUS results		
	Pml03	Pml08	Pml09	PLGT62	Putative father identification	LOD	Δ
Female DSR4423 (8)	242, 248	224, 230	216, 219	158, 158			
Embryo A	248, 254	224, 224		154, 158			
Embryo B	242, 252	230, 232		158, 158			
Embryo C	248, 254	228, 230		158, 162			
Hypothetical paternal genotype	254, 252	232, 228, 224		154,162,158			
Female DSR4436 (9)	246, 248	226, 230	249, null				
Embryo A	242, 246	224, 230	242, 249	163, 163			
Embryo B	246, 250	224, 226	249, 249	163, 163			
Embryo C	242, 246	224, 226	242, 249	151, 163			
Embryo D	242, 246	224, 226	242, null	163, 163			
Hypothetical paternal genotype	242, 250	224	242, 249	163, 151?			
Female DSR5650 (10)	246, 246	226, 228					
Embryo A	246, 250	226, 230		165, 167			
Embryo B	246, 250	228, 230	243, 247	165, 167			
Embryo C	246, 252	228, 230	243, 247	165, 167			
Hypothetical paternal genotype	252, 250	230	243 and/or 247	165 and/or 167			

[a] There are only 2 paternal alleles (not 3), but low resolution did not allow us to differentiate between 2 of them.

to estimate the most likely father for each offspring (taking into account typing error). A log-likelihood ratio score of 0 implies that a putative male is equally likely to be the father of the offspring as a randomly selected male, whereas a positive log-likelihood ratio score implies that the putative male is more likely to be the father than a randomly selected male. Statistical confidence (delta [Δ]) was estimated for critical values at both strict (95%) and relaxed (80%) confidence levels based on computer simulation of paternity inference with allele frequencies from the population under study.

Allele frequencies were estimated from 63 individuals but the remaining simulation parameters were estimated only from males (adults and juveniles) and females ($n = 51$) with embryos large enough to genotype. Thus, paternity analysis is conservative in that it considers juveniles as potential sires. In addition, CERVUS is relatively insensitive to the presence of unknown close relatives of the parents among the candidate males (Marshall et al. 1998)—an important factor because related males possibly are present in the sample. Simulation parameters were as follows: 100,000 cycles; 100% of loci typed with an error rate of 0.015; and 47 candidate parents, of which 95% were sampled. The error rate was determined by estimating the percentage of offspring–mother mismatches. To estimate the number of candidate males in the population, we used the Leslie estimate (Leslie and Davis 1939), a standard formula developed to predict the initial population size before removal trapping. The total population size was estimated to be 64 animals (we collected 63). Given the sampled sex ratio, we used a conservative estimate of 47 candidate males in the simulation.

Results

Population Characteristics

Approximately 6,200 trap nights yielded 63 *P. crinitus* (44 males and 19 females). Seven females from Stansbury Island and 3 from the other Utah localities were pregnant and carried embryos sufficiently developed to enable DNA extraction. The sex ratio of the population of *P. crinitus* was significantly male biased (2.3:1; $P < 0.05$). On Stansbury Island, pregnant females averaged 4.0 embryos per litter (range = 3–5 embryos), whereas the 3 females from other Utah localities averaged 3.3 embryos per litter (range = 3 or 4 embryos). Twenty-one percent of litters examined on Stansbury Island contained 5 embryos.

Genetic Data

Sixty-six adults and subadults and 38 corresponding embryos were genotyped at 4 highly variable microsatellite loci. Polymorphism among the loci averaged 9 alleles per locus (range = 8–10) and mean expected heterozygosity (H_E) was 0.754 ($SD = 0.030$; Table 1). Observed heterozygosity (H_O) was consistently lower at all loci ($\bar{X} = 0.674$; $SD = 0.057$), although not significantly so. The paternal exclusion probability (Jamieson 1965; Selvin 1980) across 4 loci when 1 parent was known was 0.960. The mean probability of identity (Paetkau and Strobeck 1994) across all loci was <0.001. We obtained a probability of identity for full siblings of 0.024, which is lower than values obtained and cutoff lines used in other similar studies (see Lucchini et al. 2002; Pearse et al. 2001a; Woods et al. 1999). In our data set, 2 males (QRS16 and QRS57) had identical genotypes across 4 loci. Thus, 0.032 of sampled individuals had matching genotypes, a result that is not significantly different from the expected value of 0.024 ($P = 0.61$). Thus, we feel confident that the probability of identity for full siblings estimated above is accurate in our sample and that a male genotype that fails to match a hypothetical paternal genotype reconstructed from a litter is not the actual sire.

Among the Stansbury Island samples, 2 embryos (B and C of litter 6 from female QRS55; see Table 2) at locus Pml08 did not amplify a maternal allele. We attribute the discrepancy to a null allele inherited through the mother (female QRS55), which is scored as homozygous at this locus. Amplification at locus Pml09 did not produce consistent results in litter 8 and consequently we excluded those data from our analysis. How-

TABLE 3.—The number of alleles per litter that did not correspond to the maternal genotype and hence were assigned as paternally derived. Multiple paternity assignments are based on criterion 1.

Litter	Number of paternal alleles amplified	Multiple paternity
1	≤2	No
2	≤2	No
3	≤2	No
4	3 (1 locus)	No
5	≤2	No
6	≤2	No
7	3 (1 locus)	No
8	3 (2 loci)	Yes
9	≤2	No
10	≤2	No

ever, this did not affect our conclusion with regard to the paternity of this litter.

Loci at which no alleles amplified include locus PLGT62 (females 4436 and 5650) and locus Pml09 (female 5650 and embryo A from litter 10). We suspect that the DNA from female 5650 and embryos was degraded because gel verification after extraction always resulted in very weak bands relative to other samples. DNA corresponding to problematic loci were reextracted, reprocessed, or both, at least twice and was only included in the analysis when consistent results were obtained.

Assignment of Paternity

Criterion 1.—One of 10 litters showed evidence of multiple paternity according to criterion 1 (Table 3). In litters 4 and 7, 3 paternal alleles amplified at a single locus with only 2 at all remaining loci (see results under "Criterion 2" for evidence of a null allele for QRS44). Therefore, under the parameters previously set forth, we conclude that these litters also were singly sired. Litter 8 from Uintah County amplified 3 paternal alleles at 2 loci (Table 2). Hence, we conclude that this litter was multiply sired.

Criterion 2.—Genotypes of males QRS20, QRS33, QRS49, and QRS53 each match the hypothetical paternal genotypes in a sampled litter (Table 2). In each case, the assigned male was the only male sampled whose genotype matched all paternal alleles in all corresponding embryos. The genotype of male QRS39 explains most parsimoniously all paternal alleles in litter 2, with the exception of embryo B at locus Pml08. This locus has a paternal allele (204 or 232) not found in QRS39. Four possible scenarios could explain this discrepancy: 1) the paternal allele is present but failed to amplify in QRS39 (the individual is scored as homozygous at this locus); 2) a mutation occurred at locus Pml08 in embryo B (litter 2); 3) an unsampled male in the population had the same genotype as QRS39 with the exception of being heterozygous at Pml08; or 4) QRS39 is the father of 4 embryos and another unsampled male with a genotype similar to that of QRS39 is the father of embryo B (litter 2). Given that the paternal exclusion probability was 0.960 and that we had a thorough sampling regime, options 1 or 2 are most likely. Therefore, we assigned paternity of litter 2 to male QRS39.

Two lines of evidence suggest that litter 7 was multiply sired. First, the hypothetical paternal genotype contains 3 alleles at locus Pml09. Second, although there are only 2 paternal alleles at locus PLGT62, no sampled male has a matching genotype at that locus (171 and 173). The 1st evidence alone is not sufficient to reject the null hypothesis of single paternity based on criterion 1. However, juxtaposing both lines of evidence strongly supports multiple paternity. Four males (QRS34, QRS35, QRS43, and QRS37) each have genotypes that match the inferred paternal alleles for at least 1 embryo in litter 7. QRS34 matches the paternal genotype of embryos A, B, and C; QRS37 matches that of embryo D; and QRS35 and QRS43 match paternal genotypes of embryos B and C. Thus, the most conservative conclusion is that QRS34 sired embryos A, B, and C and QRS37 sired embryo D.

Spatial Relationships of Putative Fathers

Linear distances among trap locations of mothers and putative fathers are listed in Table 4. Seven putative sires (specimen numbers QRS20, QRS39, QRS53, QRS49, QRS33, QRS34, and QRS37) were captured between 7.5 and 70 m from their respective breeding partners (QRS18, QRS22, QRS25, QRS54, QRS55, and QRS72). One of 2 males nearest to female QRS18 apparently sired her litter (litter 1), but it is not clear whether it was the closest male (13 m distant) or the male at a slightly greater distance (26 m). In either case, male QRS20 was caught in close proximity to its breeding partner. Male QRS51 was the only sire not captured on the same outcropping as the litter to which it was assigned (litter 4). The linear distance between it and female QRS44 was 174 m, well over twice the distance between any other breeding pair assigned with high confidence.

Although males QRS28 and QRS150 were captured approximately 1.3 km away from female QRS72, the 2 more likely sires (males QRS34 and QRS37) of litter 7, based on genetic data, were trapped at distances of 39 m ± 23.7 m and 55 ± 30.5 m away from female QRS72, respectively. Thus, the spatial and genetic data are concordant and, under criterion 2, there is strong evidence that males QRS34 and QRS37 sired litter 7.

Criterion 3.—Critical values of delta (Δ) were 1.32 (strict) and 0.58 (relaxed). This means that any putative fathers with values of delta greater than these thresholds have a corresponding probability ($P = 0.95$ for strict; $P = 0.80$ for relaxed) of being the actual father of the offspring to which they are assigned. One male (QRS33) was supported at the 95% confidence level as the exclusive sire of all embryos in litter 6 (Table 2). All remaining litters, except litter 1, had at least 1 male assigned to them at least above the 80% confidence level. However, none of those had a single male assigned to every embryo within a litter. The power of CERVUS to assign paternity to individual embryos was moderate to low within most litters at high (11 of 28) and relaxed (14 of 28) confidence levels. Within litter 7, male QRS34 was assigned to embryo A with more than 80% confidence, and QRS37 was assigned to embryo D (litter 7) with 95% confidence. Thus, at a relaxed confidence level, at least 1 (14%) of the 7 litters on Stansbury Island was multiply sired.

Table 4.—Results of paternity assignment for the 3 criteria combined. Criterion 1 = direct observation; criterion 2 = male genotype correlated with hypothetical paternal genotype and spatial relationship data; criterion 3 = CERVUS analysis. Question mark indicates that a male was tentatively assigned to the corresponding embryo, but with only moderate confidence. Distances between mothers and putative fathers > 30 m were estimated from global positioning system coordinates taken at the point of capture. Error (in parentheses) is a summation of the calculated error given by the global positioning system unit for each location. Parents are identified by QRS or DSR specimen numbers.

Mother identification (litter number)	Offspring identification	Paternity assignments per criterion			Distance between mother and putative father (m)
		Criterion 1	Criterion 2	Criterion 3	
QRS18 (1)	A, B, C	Single	QRS20		13−26
QRS22 (2)	A, D, E	Single	QRS39	QRS39	40 (± 11)
	B, C	Single	QRS39		
QRS25 (3)	A, C, D	Single	QRS53	QRS53	45−70 (estimated)
	B	Single	QRS53		
QRS44 (4)	A, C	Single	QRS51 ?		174 (± 11)
	B, D	Single	QRS51 ?	QRS51	
QRS54 (5)	A, C, D, E	Single	QRS49		7.5
	B	Single	QRS49	QRS49	
QRS55 (6)	A, B, C	Single	QRS33	QRS33	10
QRS72 (7)	A	Single	QRS34	QRS34	39 (± 23.7)
	B, C	Single	QRS34		
	D	Single	QRS37	QRS37	55 (± 30.5)
DSR4423 (8)	A, B, C	Multiple			
DSR4436 (9)	A, B, C, D	Single			
DSR5650 (10)	A, B, C	Single			

Combined Evidence

No assertion of paternity is inferred from a single criterion that is contradicted by results from another criterion except in 1 case within litter 7 (Table 4). Because this litter had 3 paternal alleles at only 1 locus, it was considered singly sired under criterion 1, but multiply sired under criteria 2 and 3. Not only do criteria 2 and 3 agree that litter 7 was multiply sired by QRS34 and QRS37, but these criteria also are consistent in the assignment of QRS34 to embryo A (litter 7) and QRS37 to embryo D (litter 7; Table 4). The strong correlation between results from criteria 2 and 3 coupled with the lack of resolution inherent under our conservative application of criterion 1 lead us to conclude that litter 7 was multiply sired. Therefore, we conclude, based on evidence from combined analyses, that a minimum of 2 litters (20%) from 2 Utah populations were multiply sired.

Discussion

This study provides evidence that multiple paternity occurred in at least 2 populations of *P. crinitus* in Utah during 2 breeding seasons. By using 3 methods for determining paternity, we were able to assign parentage to 8 males and we have demonstrated multiple paternity in 1 of the litters from Stansbury Island (as well as 1 from Uintah County). However, no evidence was found that any male bred with more than 1 female during a single breeding season (albeit our sample size was small). We estimate that the minimum frequency of multiple paternity was 20% among the total sampled litters in Utah. This is consistent with results for other *Peromyscus* species in which multiple paternity has been detected (10–40% in *P. maniculatus* [Birdsall and Nash 1973; Ribble and Millar 1996], and at least 25% in *P. leucopus* [Xia and Millar 1991]) and supports the prediction of Dewsbury (1981) and our original hypothesis that multiple paternity occurs in *P. crinitus*. Additionally, examination of spatial data among breeding partners indicates that males generally remain in close proximity to an impregnated female during the gestational period.

Variation among different mating behaviors, both social and genetic, within species and populations has been well documented (e.g., Crim et al. 2002; Gibbs et al. 1994; Kelly et al. 1999; Xia and Millar 1991). Reynolds (1996) concluded that with the blurring of distinctions among breeding systems caused by a deluge of new data, it may be better to simply describe variation among individuals rather than trying to characterize species within the narrowness of a defined mating system. The fact that we found no evidence that a male sired offspring in more than a single litter should be taken as a description of individual reproductive results (or lack of data) and not a generalization of the entire population. Ideally, reproductive success should be examined for the lifetime of organisms within a population in order to detect variation in behavior and success at different stages of an individual's lifetime (Clutton-Brock 1988). One must always interpret with caution any paternity study from a single year because reproductive success may fluctuate significantly between years (Millar et al. 1994; Pearse et al. 2001b). Had our study been extended over a longer period of time, across several populations, and included more samples, we would have developed a more complete picture of the mating system of *P. crinitus*. However, because we demonstrated multiple paternity in 2 separate populations of *P. crinitus* that were collected in different years, it is highly likely that multiple paternity generally occurs in this species. This is an important finding because it contributes to our limited knowledge of the behavior and ecology of *P. crinitus* and to the growing body of literature documenting mating systems within *Peromyscus*.

The low level of multiple paternity that we documented on Stansbury Island is not surprising given the low productivity of the habitat. A dispersed social organization would be expected in a species that inhabits unproductive, arid habitat, and we would expect that encounters between males and receptive females would be significantly lower in such a habitat than in high-density populations. A detailed study of home-range size and population density would add important insights to the results of this study.

Several key differences in methods between criteria 2 and 3 were used to analyze the data. Paternity assignment under criterion 2 was accomplished by manually examining and comparing male genotypes to inferred paternal genotypes without regard to population allelic frequencies. For example, if 1 male could account for paternal alleles in embryos A–D and a 2nd male could account for paternal alleles in embryo D, then paternity was assigned to the 1st male for all 4 embryos. Thus, criterion 2 is a holistic approach that considers all embryonic genotypes within a litter simultaneously rather than solely on an individual basis. Consequently, results are inherently conservative. Under maximum likelihood, CERVUS takes into account allele frequencies and, when assigning paternity, weighs more heavily those alleles that are rare in the population. Each embryo is analyzed independently with no regard for male assignment to other embryos within the same litter. CERVUS also takes into account typing error, estimated null allele frequency, and proportion of candidate males sampled. Criterion 2 is not designed to consider any of these estimates, but rather analyzes the genotypes at face value. The benefit of including criterion 2 is that it not only allows for increased confidence in paternity assignment through an additional verification of CERVUS, but it also gives a conservative or lower limit of multiple paternity frequency within the population.

Genotypic data of males coupled with spatial data (criterion 2) allowed a greater number of paternity assignments to be made and with greater confidence than would have been possible if only CERVUS results had been analyzed. Johnson (1976) estimated home-range size for male *P. crinitus* in southern Utah at 0.371 ha (3,710 m^2; no significant difference was found between home-range sizes of 7 males and 6 females). Home ranges are by no means restricted to square dimensions, but for the sake of reference, a square of 3,710 m^2 has dimensions of about 60 $\times$ 60 m. Therefore, it is unlikely that males that were captured several hundred meters away from a pregnant female and have a matching genotype for some but not all embryos are in fact the actual fathers. This assertion is supported by the fact that all but 1 male ($n = 7$) that were assigned with high probability by CERVUS to a litter were found within the same rock outcropping and within 70 m from the mother. (Recall that male QRS51 was captured in an adjacent outcropping 174 m away from litter 4). It is interesting to note that no male was assigned sireship of a litter on the opposite ridge at a distance of $>$1 km. Thus, our tri-criterion not only allowed us to document behavior of males shortly after copulation, but also allowed for greater confidence in paternity assignments. Because CERVUS is sensitive to a decrease in the proportion of candidate males sampled (Marshall et al. 1998), combining these methods could prove to be more beneficial than an analysis using CERVUS only, especially for species whose life history would make it difficult to collect a large portion of candidate males.

Acknowledgments

We thank M. Kalcounis-Rüppell for providing aliquots of primer pairs for optimization and Nicole Lewis-Oritt for help with fieldwork. J. W. Sites, Jr., D. A. McClellan, members of the Idaho State University "Topics in Molecular Ecology" seminar, and 2 anonymous reviewers offered helpful comments on earlier drafts of this manuscript. D. K. Shiozawa and D. Gonzalez assisted with ecological aspects of this study. Credit is due to fellow graduate students in the D. McClellan and D. Rogers laboratories who were supportive through all aspects of this study. Financial support for this project came from the Monte L. Bean Life Science Museum at Brigham Young University, Provo, Utah, and National Science Foundation award DEB-98-15881 to J. W. Sites, Jr. (for support of DEP).

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Submitted 6 May 2004. Accepted 29 September 2004.

Associate Editor was Enrique P. Lessa.

APPENDIX I

Specimens examined.—Voucher specimens ($n = 54$) are housed in the Monte L. Bean Life Science Museum (MLBM), Brigham Young University (BYU), and include all sampled individuals of *P. crinitus* (not just those used in the paternity analyses). All specimens are from Utah. Data are listed in the following format: collecting locality (ordered by Utah counties), elevation, MLBM voucher number(s), collector catalog number(s), sex (M = male, F = female). Some individuals were captured at the same locality and are listed together. Nonpregnant and nongenotyped females are not included, but are housed in the MLBM and can be made available upon request from D. S. Rogers.

Kane County: 59 km E, 25 km N, Kanab, 1,450 m, BYU18065, DSR5650 (F).

Tooele County (all from Stansbury Island): 40°47.534′N, 112°31.506′W, 1,320 m, BYU18789, BYU18790, QRS16 (M), QRS17 (M); 40°48.229′N, 112°30.967′W, 1,407 m, BYU18791, BYU18792, BYU18793, BYU18794, QRS18 (F), QRS19 (M), QRS20 (M), QRS22 (F); 40°48.250′N, 112°30.721′W, 1,565 m, BYU18796, QRS25 (F); 40°47.674′N, 112°31.167′W, 1,547 m, BYU18797, QRS26 (M); 40°48.306′N, 112°30.707′W, 1,565 m, BYU18798, BYU18799, QRS27 (M), QRS28 (M); 40°47.654′N, 112°31.054′W, 1,583 m, BYU18801, QRS30 (M); 40°48.309′N, 112°30.697′W, 1,566 m, BYU18802, QRS31 (M); 40°48.248′N, 112°30.911′W, 1,455 m, BYU18803, QRS32 (M); 40°47.651′N, 112°31.105′W, 1,540 m, BYU18804, QRS33 (M); 40°47.680′N, 112°31.077′W, 1,553 m, BYU18805, QRS34 (M); 40°47.676′N, 112°31.070′W, 1,561 m, BYU18806, QRS35 (M); 40°47.611′N, 112°31.100′W, 1,508 m, BYU18807, QRS36 (M); 40°47.647′N, 112°31.092′W, 1,549 m, BYU18808, QRS37 (M); 40°47.687′N, 112°31.122′W, 1,550 m, BYU18809, QRS38 (M); 40°48.235′N, 112°30.941′W, 1,426 m, BYU18810, QRS39 (M); 40°47.674′N, 112°31.176′W, 1,538 m, BYU18811, QRS40 (M); 40°47.701′N, 112°31.153′W, 1,544 m, BYU18812, QRS41 (M); 40°47.640′N,

112°31.129′W, 1,535 m, BYU18813, QRS42 (M); 40°48.228′N, 112°30.687′W, 1,565 m, BYU18814, QRS43 (M); 40°48.222′N, 112°30.819′W, 1,508 m, BYU18815, BYU18821, QRS44 (F), QRS50 (M); 40°48.223′N, 112°30.829′W, 1,561 m, BYU18816, QRS45 (M); 40°48.228′N, 112°30.707′W, 1,549 m, BYU18817, QRS46 (M); 40°48.228′N, 112°30.687′W, 1,565 m, BYU18818, BYU18823, QRS47 (M), QRS52 (M); 40°48.235′N, 112°30.917′W, 1,442 m, BYU18819, QRS48 (M); 40°48.229′N, 112°30.950′W, 1,420 m, BYU18820, QRS49 (M); 40°48.234′N, 112°30.943′W, 1,426 m, BYU18822, QRS51 (M); 40°48.250′N, 112°30.721′W, 1,522 m, BYU18824, QRS53 (M); 40°48.232′N, 112°30.948′W, 1,420 m, BYU18825, QRS54 (F); 40°47.647′N, 112°31.092′W, 1,549 m, BYU18826, QRS55 (F); 40°48.243′N, 112°30.944′W, 1,433 m, BYU18828, QRS57 (M); 40°48.223′N, 112°30.939′W, 1,421 m, BYU18829, QRS58 (M); 40°47.630′N, 112°31.147′W, 1,544 m, BYU18833, QRS62 (M); 40°47.680′N, 112°31.077′W, 1,553 m, BYU18834, QRS63 (M); 40°48.308′N, 112°30.691′W, 1,562 m, BYU18835, QRS64 (M); 40°47.701′N, 112°31.153′W, 1,544 m, BYU18836, QRS65 (M); 40°47.635′N, 112°31.124′W, 1,552 m, BYU18838, QRS70 (M); 40°47.691′N, 112°31.064′W, 1,570 m, BYU18839, QRS71 (M); 40°47.675′N, 112°31.104′W, 1,557 m, BYU18840, QRS72 (F); 40°48.248′N, 112°30.911′W, 1,455 m, BYU18842, QRS149 (M); 40°48.295′N, 112°30.742′W, 1,502 m, BYU18843, QRS150 (M); 40°47.674′N, 112°31.167′W, 1,532 m, BYU18946, QRS153 (M); 40°48.221′N, 112°30.820′W, 1,515 m, BYU18936, BYU18847, BYU18848, QRS159 (M), QRS160 (M), QRS161 (M).

Uintah County (Bitter Creek Canyon): 39°40′05″N, 109°14′W, 1,860 m, BYU16641, DSR4423 (F); 39°37′35″N, 109°11′30″W, 1,920 m, BYU16631, DSR4436 (F).