



Social but not genetic monogamy is associated with greater breeding success in prairie voles

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Much attention has focused on distinguishing between social and genetic monogamy in avian taxa. However, surprisingly few studies have directly investigated this distinction among mammals. We investigated the genetic mating system of the prairie vole, *Microtus ochrogaster*, a popular model for mammalian monogamy and human attachment. We used space use patterns to define paired and single animals and assessed paternity using microsatellite loci. Prairie voles in this study engaged in significantly more extrapair fertilizations than predicted under genetic monogamy but fewer than predicted under random mating, demonstrating social but not genetic monogamy. Furthermore, we found that paired individuals were more likely to produce offspring than were unpaired individuals of either sex. This finding was true for both sexes and was attributable to differences in fertilization rates rather than litter sizes. Among mated individuals, however, faithful animals were no more successful than those that mated outside a pair. Taken together, our data demonstrate that paired prairie voles have greater breeding success than single voles, but such success is not contingent on mating exclusively with a social partner. If this species is to serve as a model for human love, our findings emphasize the need to distinguish between mammalian social attachment and sexual fidelity.

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Explanations for the origins of monogamy often emphasize the distribution of females in space and time (Emlen & Oring 1977; Komers & Brotherton 1997; Shuster & Wade 2003) or the need for biparental care (Trivers 1972; Kleiman 1977). Both explanations are indebted to a classic study that asserts that male fitness increases with multiple mates, whereas female fitness does not (Bateman 1948; but see Tang-Martinez & Ryder 2005). These paradigms have been powerful tools within the avian literature (e.g. Westneat et al. 1990; Birkhead & Møller 1992) but have been less explored among mammals (Reichard 2003). Among rodents, both female spatial

distributions and male parental care have been offered as explanations for monogamy in the few species studied to date (Foltz 1981; Gubernick & Teferi 2000; Reichard 2003; Ribble 2003).

Despite its importance, space use (or cohabitation) alone is an insufficient descriptor of mating system. For example, seemingly monogamous species of birds rarely exhibit sexual fidelity, a finding that ought to generalize to other groups (Westneat et al. 1990; Birkhead & Møller 1992; Reichard 2003). Researchers now distinguish between social monogamy, defined by an exclusive living arrangement, and genetic monogamy, in which cohabitation is accompanied by exclusive parentage (Gowaty 1996; Reichard 2003). Nevertheless, such distinctions in mating tactics have rarely been directly assessed in mammalian taxa (however, see Goossens et al. 1998). Interestingly, both of the monogamous North American rodents whose genetic and social mating systems have been well

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characterized, *Peromyscus californicus* and *Peromyscus polionotus*, exhibit genetic monogamy (Foltz 1981; Ribble 1991, 2003). Furthermore, breeding pairs of the philopatric pine vole, *Microtus pinetorum*, also appear to engage in both social and genetic monogamy in the field (Marfori et al. 1997).

Monogamy is uncommon among mammals, occurring in less than 3% of species (Kleiman 1977). Perhaps the best-known example of a nonhuman mammalian species to engage in monogamy is the prairie vole, *Microtus ochrogaster*. In natural settings, most adults form long-lived male–female pairs (Getz et al. 1981, 1993; Getz & Hofmann 1986; Getz & Carter 1996). In the laboratory, pairs commonly show strong affiliative preferences for each other (e.g. Williams et al. 1992), animals that lose a mate are unlikely to pair with another (Pizzuto & Getz 1998), and pairs that have been separated show behavioural profiles considered markers of depression (Bosch et al. 2005). Given such data, the prairie vole has become a popular model for both the ecological contexts of monogamy (e.g. Getz & Carter 1980, 1996; Getz et al. 1981, 1993) and the molecular and neural substrates of social attachment or pair bonds (e.g. Carter 1998; Young & Wang 2004). Indeed, the prairie vole pair bond has emerged as a model for human love (Carter 1998; Young & Wang 2004). Whereas extensive field and laboratory work has investigated monogamous behaviour in this model species, no complete investigation of prairie vole mating fidelity has been reported. Getz & Carter (1996) anecdotally noted that prairie voles might mate outside the pair bond based on observations in laboratory studies (Carter et al. 1995; Wolff & Dunlap 2002); however, they describe that DNA fingerprinting of embryos of free-living male–female pairs revealed exclusive mating between partners, even at high population densities (Getz & Carter 1996). Solomon et al. (2004) reported that five of nine pregnant females trapped in the wild bore litters with more than one sire. In Solomon's study, however, whether these females were single or paired was unknown; thus, although suggestive of unfaithful mating in a monogamous species, this study was unable to assess whether a breeding partnership existed and therefore could not determine whether the multiple mating represented infidelity between partners or multiple mating by single females with wandering males (cf. McGuire et al. 1990; Getz & McGuire 1993; Lyons & Getz 1993). Given the status of the prairie vole as a model system for monogamy, not only is it imperative to compare space use and paternity in natural or seminatural environments but also surprising that no such study has previously done so.

Although prairie voles are commonly cited for their monogamous behaviour, there are individual differences in the pairing status of adult animals. Most 'resident' adults form life-long pairs, cohabitate and provide biparental care, but as many as 35–45% will adopt a non-territorial, 'wandering' phenotype at some point in their lives (Thomas & Birney 1979; Getz et al. 1993). Wandering males may be either particularly attractive males that can forgo mate guarding or unattractive males that cannot pair and are making the 'best of a bad job' (Getz & McGuire 1993; Getz et al. 1993; Solomon & Jacquot

2002). No data effectively distinguish between these hypotheses. Indeed, we lack a definitive examination of the genetic mating system of this species. By investigating components of fitness, such as likelihood of fertilization, in combination with information about pairing status (informed by space use) we can provide a rigorous description of the genetic mating system and begin to assess the benefits of social and genetic monogamy in a monogamous mammal. Here we use a combination of radiotracking and paternity analyses on captive-reared animals released into seminatural enclosures to investigate the modal mating system and the reproductive consequences of the individual mating tactics that make up the prairie vole mating system. We make the following predictions: (1) if prairie voles are genetically monogamous, then paired animals should not produce extrapair offspring, (2) if prairie voles are socially monogamous, then a majority of male–female pairs should have significant spatial overlap with each other and minimal overlap with other opposite-sex conspecifics, (3) if being paired is fitness enhancing, then paired animals should have greater reproductive success than single animals and if being single (e.g. a wandering male) is fitness enhancing, then the opposite should be true and (4) if mating exclusively with a partner is fitness enhancing, for any reason, then inpair fertilizations should outnumber extrapair fertilizations in both sexes. Here, we test these predictions and discuss the implications of these results on the current use of the prairie vole as a model of social attachment and human love.

METHODS

Test Animals

We used 48 male and 48 female outbred prairie voles. All individuals were derived from wild-caught populations from either Shelby County, Tennessee (TN) or Champaign County, Illinois (IL) one to three generations prior to the experiment. These animals were bred at the University of Memphis Animal Care Facility and weaned at 21 days. At weaning, we grouped all animals into same-sex littermates and housed them together in polycarbonate cages (29 × 18 × 13 cm); there were two to four sibs per cage. No singly housed animals served as subjects in this study. Standard rodent chow (Harlan Teklad, Madison, WI, U.S.A.) and water were provided ad libitum, temperature was maintained at 21 ± 2°C, and photoperiod was maintained under a 14:10 h light:dark cycle. Housing arrangements allowed animals to have visual and olfactory but not tactile contact with noncagemate conspecifics.

Field Methods

We allowed animals to establish territories and reproduce freely in seminatural enclosures to assess patterns of space use and to compare the breeding success of individuals that were pair bonded and single. We distributed animals into eight groups, each consisting of six unrelated adult males and females. All animals were sexually mature

but inexperienced and all were of similar age (male: 88.3 ± 4.35 days; female: 96.1 ± 4.65 days) and weight (male: 36.9 ± 1.13 g; female: 34.8 ± 0.88 g). Animals in each group were from the same geographical origin. All individuals were collared with a 1.9-g unique-frequency-emitting transmitter (BD-2C; Holohil Systems Ltd, Carp, Ontario, Canada), eartagged and weighed, and a tail clipping was taken before the experiment was initiated (see [Ethical note](#)). Based on the results of [Getz & McGuire \(1993\)](#), we assumed that single females would readily establish nests, whereas single males would wander until encountering a female nest site, which they would later join. Allowing females to find shelter before introducing males increased our confidence that females would not be distracted by searching for shelter while simultaneously making mate choice decisions. Therefore, we introduced females of each group into seminatural enclosures 2 days before males to allow them to establish territories and find shelter. [Getz et al. \(1993\)](#) report that population densities range from 11 to 624 voles/ha. The population densities we created (200 voles/ha) were above the criteria for distinguishing 'low' and 'high' densities used by [Getz et al. \(1993\)](#); either $<$ or $>$ 100 voles/ha, but they fell well within the limits of these natural population densities that both [Getz et al. \(1993\)](#) and others (e.g. [Taitt & Krebs 1985](#)) have observed. Furthermore, [Getz et al. \(1987\)](#) report that prairie vole monogamy is independent of population density.

The four study enclosures each measured 20×30 m and were located in Shelby County, TN, U.S.A. (see [Mahady & Wolff 2002](#); [Ophir et al. 2007](#)). Each enclosure was fitted with polyvinyl chloride piping and electric wire along the top to deter climbing by voles or terrestrial predators and perching by predatory birds. Vegetation within each field enclosure consisted primarily of mixed pasture grasses (e.g. rye, fescue and brome) and dicots suitable to sustain prairie vole populations. Of the eight groups, four were composed exclusively of animals from an IL lineage whereas the other four groups consisted entirely of animals derived from TN stock. Concurrently running two groups (one from each region) at a time, we ran all eight groups of voles (one group per enclosure) over the 2004 breeding season (July–October). We reused enclosures from one run to the next, counterbalancing which population a group was composed of across each enclosure used. Using a nested ANOVA design to control for within- and between-enclosure variances, we found no notable behavioural or morphological differences between the two populations ([Ophir et al. 2007](#)). Furthermore, a series of laboratory studies independently revealed no behavioural or morphological differences between voles from these two sites ([Ophir et al. 2007](#)); we, therefore, combined data from the two populations.

Radiotracking began 4 days after females were introduced into the empty enclosures. We radiotracked subjects twice daily for 12–14 days along a 4×5 grid with 4-m spacing to assess spatial distribution. We varied the time at which we recorded fixes across the day during daylight hours (between 0600 and 2000 hours) to avoid problems associated with temporally dependent habits. Fixes

collected on the same day were separated by a minimum of 30 min, and by a mean \pm SE of 5.8 ± 0.32 h. To guarantee that all mothers were known (see [Parentage analysis](#) below), we ensured that no females gave birth before trapping by removing all animals 18–20 days following the introduction of males (gestation is approximately 21 days; [Gier & Cooksey 1967](#)). Thus, the experiment spanned pair bond formation and pregnancy but ended before birth of the young. Trapping before birth also enabled us to focus on a single standard component of fitness (i.e. fertilization success) by removing any subsequent effects of either bi- or uniparental care. In addition, focusing on the number of embryos allowed us to remove the variance in offspring survivability that might result from variables we could not control.

We defined pairs as individuals who overlapped each other's home range more than they overlapped the home ranges of all other individuals combined (see below). To estimate the size of each individual's home range, we used RANGES V (Anatrack Ltd, Dorset, U.K.) to calculate minimum convex polygons (MCP) based on location fixes from the assembled X and Y coordinates. MCP estimates are the simplest and most common measures of space use. However, when estimating encounter rates between individuals, they can substantially overestimate interactions in the outer margins of the home range by assuming that an animal is equally likely to be anywhere in its range. One solution is to use kernel methods. However, kernel estimators are problematic because they make some restrictive assumptions about the distribution of the data, they require large data sets, they are often difficult to compare between studies because smoothing factors vary and are rarely reported in the literature and they can lead to biased measures of space use ([White & Garrott 1990](#); [Powell 2000](#); [Row & Blouin-Demers 2006](#)). Another common solution is to reduce the number of locations used to calculate the MCP for an animal so that the area contained within the MCP represents the area of an animal's home range that it is most likely to occupy (its core home range; [White & Garrott 1990](#)). To choose an appropriate level of fix exclusion, we fitted a curve to the average peeled polygon home ranges, which removed the locations furthest from the harmonic mean centres of the home ranges at 5% intervals. The point at which the outermost locations no longer biased the MCP estimate of home ranges, or where the slope switched from being steep to shallow, fell between 70 and 80% cores (where Y equals MCP area, and X equals the locations included in cores at 5% intervals; $Y = -29.0 \ln(X) + 88.15$; $r^2 = 0.99$). We therefore chose 75% cores to describe the animals' area of primary space use. However, we did not find our results to be strongly influenced by changes in this criterion, for example by 95% cores, which is a common but arbitrary parameter used for MCP analysis ([White & Garrott 1990](#); [Kenward 2001](#)).

We used MCP estimates of core home range to distinguish paired (resident) and single (wandering) males. Thus, we calculated the pairwise encounter estimates (PE) by taking the product of the proportion of home range overlap between all possible pairs in an enclosure (including animals that did not overlap as 0% overlap).

$$PE = \frac{(\% \text{overlap male}_i | \text{female}_j) \times (\% \text{overlap female}_j | \text{male}_i)}{100} \quad (1)$$

We then divided each product by the sum of all other opposite-sex individuals, obtaining a value representing the relative encounter rate (ER) of one individual with another.

$$\text{For each individual } (i), ER_i = \frac{PE_i}{\sum_{k=1}^n PE_k} \quad (2)$$

This value represents an estimated probability of a dyadic encounter between a pair of individuals based on the proportion of home range they shared given all other individuals in the population. A relative encounter rate equal to or greater than 0.5, for example, indicates that a given male was likely to encounter a given female more frequently than all other females combined. If a male and female both demonstrated relative encounter rates of 0.5 or greater for each other, we considered them to be a pair (e.g. see Fig. 1a–d).

Parentage Analysis

Tissue collection, embryo harvesting and DNA extraction

Immediately following the recovery of test animals, subjects were brought into the laboratory and euthanized, and we collected tissue samples from all pregnant females ($N = 29$) and embryos ($N = 133$). For all males ($N = 48$), we used either tail clippings taken prior to introduction to enclosures or tissue collected at recovery to extract DNA. All samples were stored in 70% ethanol and frozen at -70°C . To harvest embryos, we extracted each fetus from the mothers' uterine horns, placed each embryo on a clean (DNA-free) surface, removed the embryonic sac and placenta, measured the crown–rump length, placed them in 70% ethanol and stored them at -70°C . Litter sizes ranged from one to seven embryos and had a mean \pm SE of 4.43 ± 0.23 . All tissue samples were thawed and DNA was extracted following standard Qiagen DNEasy spin-column protocols (Qiagen Inc., Valencia, CA, U.S.A.).

Microsatellite loci

Four microsatellite loci previously screened in other voles (MSMM-6 and MSMM-2: Ishibashi et al. 1999; MOE-2: Van de Zande et al. 2000; AV-13: Stewart et al. 1998) were amplified with fluorescently labelled primers by standard three-step polymerase chain reaction with annealing temperatures ranging from 52 to 58°C . Products were sized with ROX 400 size standard on an automated sequencer (Applied Biosystems, Foster City, CA, U.S.A., Model 310) using Genescan software. Two observers (A.B.S. and A.G.O.) independently confirmed the assigned fragment sizes and recorded genotypes for all individuals. Population subsets of unrelated adults derived from both geographical trapping areas (IL: $N = 16$; TN: $N = 17$) were tested for Hardy–Weinberg equilibrium and linkage disequilibrium using GENEPOP 3.1c (Raymond & Rousset 1995). We also amplified MSCRB-6 (Ishibashi et al. 1997),

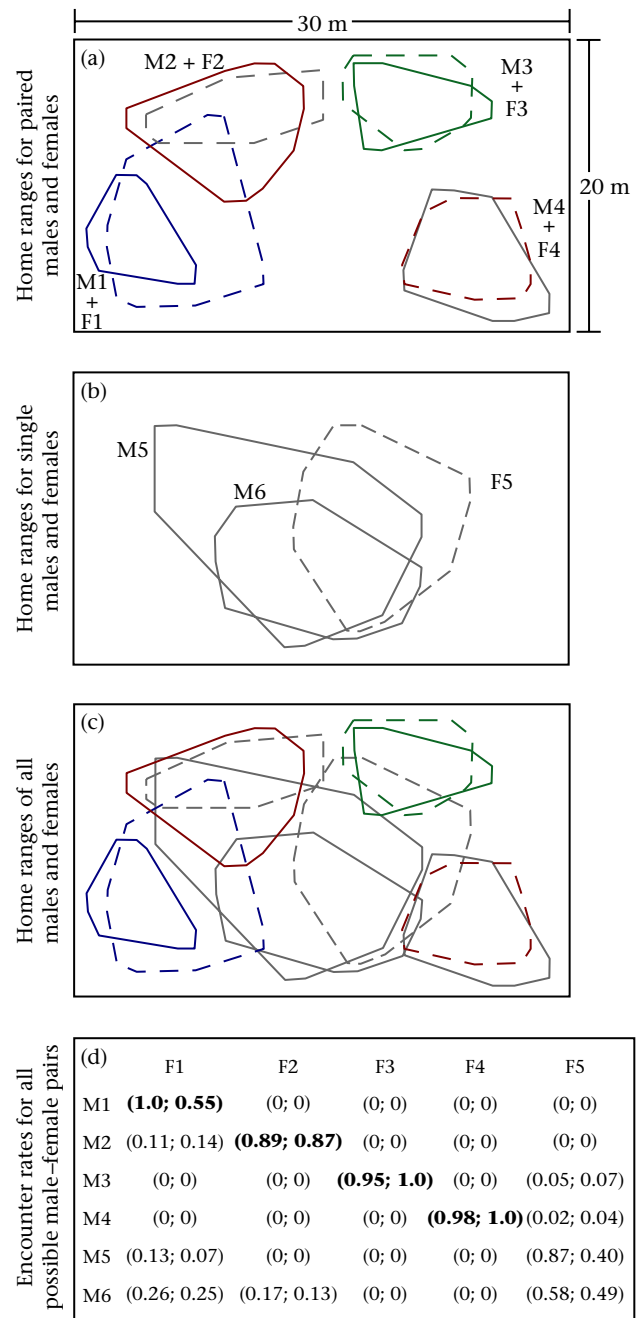


Figure 1. An exemplar distribution of 75% core minimum convex polygon home ranges in an enclosure for male (solid lines) and female (dashed lines) prairie voles. (a) Includes home ranges of paired individuals only. (b) Includes home ranges of unpaired individuals only. (c) Includes home ranges of all individuals in the enclosure. Matched colours represent individuals that had offspring together; grey represents nonreproductive animals. (d) Male-to-female and female-to-male encounter rate values (respectively) used to determine pair bond status. Bold text represents individuals that qualified as 'paired' based on our criteria. We note that the encounter rates for male M6 and female F5 closely bordered the criteria that we used to determine pairs. However, in this unique case, each animal appeared to have its own distinct nest site at opposite ends of their respective distributions, adding confidence to holding criteria so strictly.

which has been used in another analysis using prairie voles (Solomon et al. 2004). We excluded this fifth locus, however, because it yielded multiple homozygous mismatches between known maternal–offspring pairs, potentially indicating a high frequency of null alleles.

Paternity assignment

We assigned paternity to embryos using CERVUS 2.0 (Marshall et al. 1998). For each embryo, there were no more than six candidate fathers. To assign paternity, at least three typed loci were required per individual, the error rate was assumed to be 0.001%, confidence intervals were placed at 80 and 95% and simulations were run for 10 000 cycles. We considered only paternity assigned at the 95% confidence interval reliable enough to attribute fatherhood to a candidate male. Finally, we accepted paternity assignments only if the delta values (\log_e likelihood ratio of most likely to second-most likely father) were equal to or greater than 0.69, corresponding to the value at which the most likely father was at least twice as likely as the second-most likely father. Omitting this latter criterion would have caused some equivocally assigned paternities to be assigned with inflated confidence and would tend to overestimate the abundance of extrapair fertilizations and multiple paternity. By combining these data with our space use information, we could estimate the number and nature of successful matings (e.g. Fig. 1a–d).

Data Analysis

Modal mating system

To examine the modal genetic mating system, we quantified the number of individuals engaging in strictly intrapair fertilizations (IPF individuals) or at least one extrapair fertilization (EPF individuals). We then compared these frequencies to values predicted by either genetic monogamy or random mating using a replicated goodness-of-fit test (G test; Sokal & Rohlf 1981). We chose a replicated G test because it allowed us to assess departures from null expectations for IPF and EPF frequencies as well as enclosure effects on these frequencies.

We calculated the main effect G statistic by pooling data across enclosures. To calculate the between-enclosure heterogeneity statistic, we subtracted this main effect from the sum of individual G statistics for each enclosure (Sokal & Rohlf 1981). The heterogeneity statistic is a measure of how much variation is attributable to between-enclosure differences.

Although the G statistic can be assessed with a chi-square distribution, the samples within each enclosure were too small to use this approximation. To compare observed frequencies of IPF and EPF individuals to a null expectation generated by random mating, we generated null distributions by randomization, as suggested by Sokal & Rohlf (1981). We randomly reassigned fertilizations among individuals within an enclosure but limited the reassignments so that no one animal was assigned more than two fertilizations, a limitation consistent with our observed data. This method of reassignment ensured that our statistics were not biased by unnaturally high

numbers of fertilizations per individual in the randomized samples. (Excluding this limitation did not change the resulting pattern of significant effects.) We repeated the randomization 10 000 times to generate a null distribution for the G statistics.

Genetic monogamy, with its expectation of 0 EPFs, cannot be easily treated with a G test because it produces a statistic with 0 in its denominator. We note, however, that we could not detect significant heterogeneity in the frequency of IPF females in the above analysis (see Results). We did detect enclosure effects in the frequency of male IPFs, but all enclosures were biased towards higher rates of male and female IPFs than predicted under random mating (see Results). Because pooling these data would bias the outcome towards a false negative (failing to reject genetic monogamy) and there were no clear alternative statistical methods, we felt that this justified pooling data across enclosures. To test for genetic monogamy, we used a Fisher's exact test in which the null expectation was 100% IPFs.

Reproductive success

To provide an indirect measure of the fitness consequences of individual differences in mating tactic, we assessed whether paired and single animals showed a significant difference in two related measures of mating success: the probability of producing embryos and the mean number of embryos produced. Similarly, to assess fitness consequences of sexual fidelity, we determined whether animals that engaged exclusively in either within-pair or extrapair fertilizations had significant differences in the number of embryos they produced. For both sets of analyses, we averaged each measure within a sex for each enclosure, computed the across-enclosure mean and took the difference between paired and single animal means. The null distributions for these differences were generated by randomly reassigning the status of individuals within an enclosure (paired versus single, IPF versus EPF) and calculating differences in means after randomization. This approach uses the enclosure as the unit of analysis without making assumptions associated with a parametric test. Because the enclosure mean is a single datum, inferences based on across-enclosure effects do not require assessing between-enclosure heterogeneity. Through these analyses, we were able to compare paired and single animals, or IPF and EPF animals, and thus to assess how social and genetic monogamy contribute to mating success in our experimental conditions.

Ethical Note

All animals were eartagged, radiocollared and tail-clipped prior to introduction to seminatural enclosures. Standard small mammal eartags (S. Roestenburg, Riverton, UT, U.S.A.) uniquely identified each animal. We used a standard procedure to affix the aluminium eartags (2×4 mm) by piercing the pinna of the outer ear with a pointed end of the tag and securing the tag by threading the point through a hole in the back and folding the point flat. Eartag weight is negligible, discomfort during piercing

is minimal and transient and vole behaviour is not observably altered by such eartagging. Similarly, radiocollaring is noninvasive and nonrestrictive. Radiocollars were secured by threading a small cable tie (101.6 × 2.5 × 0.9 mm) through the transmitters and around subjects' necks. To facilitate free movement, we trimmed the excess from the fastened cable ties and from the antennae on the transmitters to their base. Trimming antennae in this fashion did not prevent our receiver from picking up a transmitter's signal from a distance of at least 100 m. After collaring, subjects were housed individually and monitored for a minimum of 2 days to ensure that collars were not too tight or obviously too loose and that each subject was not disturbed by the radiocollar. All animals habituated to the radiocollars in less than 3 h and behaved normally. Anticipating that some males may die before recovery, we ensured that all males were genotyped by collecting a tail clipping prior to introducing the animals into the enclosures. To minimize discomfort we applied a topical anaesthetic (bupivacaine) to the end of the tail. Using surgical scissors, we took a 2- to 3-mm clip of tissue from the tip of the tail, which was placed in 70% alcohol and refrigerated at 4°C. All animals recovered and were behaving normally within minutes of the procedure.

As mentioned above, we euthanized animals soon after trapping in the field. To collect tissue for DNA genotyping for this study and to collect brains from these animals for a complementary study (unpublished data), we sacrificed subjects with CO₂ followed by rapid decapitation. The aforementioned techniques are in line with the guide to animal care for U.S. Department of Agriculture covered species, were approved by both the University of Florida Institutional Animal Care and Use Committee (IACUC) board (animal use protocol D289) and the University of Memphis IACUC board (animal use protocol 0012) and are consistent with the ASAB/ABS Guidelines for the use of animals in research (ASAB/ABS 2006).

RESULTS

Of the 48 males and 48 females in this experiment, we recovered 43 males and 38 females (remaining animals died before recovery). Twenty-nine of the 38 recovered females were pregnant. We had ample home range data for all but two females (one of which was pregnant) who shed their radiocollars during radiotracking. We therefore excluded them from the study. Of the 36 females used in this study, we were able to determine whether the majority of embryos of 26 females were the result of an IPF or EPF; in most cases the entire litters were genotyped (see below). One male was considered paired to an omitted female that died just before trapping. We found no enclosure effects (and therefore no effects of time of season) for total body length (nested ANOVAs: $F_{7,69} = 1.45$, $P = 0.20$), weight at introduction ($F_{7,69} = 0.20$, $P = 0.98$), or home range size ($F_{7,69} = 1.24$, $P = 0.29$). As mentioned in the [Methods](#), we did not assess enclosure effects for the dependent variables in which we treated the enclosure mean as an individual datum.

A majority of males and females were considered paired (males: 32 of 43, 74.4%; females: 31 of 36,

86.1%). The mean ± SE percentage overlap of paired males (91.3 ± 2.6%) and females (87.9 ± 2.8%) was larger than the mean percentage overlap of nonpaired males (3.6 ± 0.8%) and females (4.0 ± 0.9%; Student's *t* test: males: $t_{216} = 40.45$, $P < 0.0001$; females: $t_{216} = 34.05$; $P < 0.0001$), adding confidence to our ability to reliably identify pairs. Because we did not want to disrupt the voles, we did not attempt to locate nest sites; however, many of the nest sites could be observed passively while radiotracking. Individuals that we later considered paired were commonly located in the same nest sites although not necessarily at the same time (cf. Gruder-Adams & Getz 1985). Individuals that we labelled paired were found within a 10-m radius of each other more often than all other nonpaired individuals of the opposite sex (mean ± SE number of times that paired animals were within 10 m of each other: 16.0 ± 1.03; mean ± SE number of times that nonpaired animals were within 10 m of each other: 4.1 ± 0.28).

DNA and Microsatellites

Mean ± SE allelic richness across loci was 13.8 ± 3.17 alleles (Table 1). All four loci were in Hardy–Weinberg equilibrium ($\alpha = 0.05$), and no significant linkage disequilibrium was found among them (Bonferroni correction for multiple tests: $\alpha = 0.008$). Observed heterozygosities for all samples ($N = 184$) were calculated using CERVUS (Marshall et al. 1998) and ranged 0.37–0.87 (see Table 1). We note that a similar series of analyses revealed no differences between animals derived from IL or TN in allelic richness or heterozygosity (Ophir et al. 2007). Furthermore, the proportion of males and females that bred successfully was not significantly different for IL or TN males (Fisher's exact test: IL_{Sires} = 14, IL_{Nonsires} = 10; TN_{Sires} = 12, TN_{Nonsires} = 7, $P = 1.0$) and females (IL_{Dams} = 15, IL_{Nondams} = 5; TN_{Dams} = 14, TN_{Nondams} = 4, $P = 1.0$), further justifying our treating these data as homogeneous.

CERVUS estimated an error rate of 0.0018 between maternal and embryo alleles, validating the 0.001 a priori error rate used in simulations. Paternal exclusion probability was 0.987, with mothers known. We determined that all loci were free of null alleles (CERVUS: frequency estimates < 0.05; Table 1) with the possible exception of

Table 1. Summary data for microsatellite loci used to assign paternity for all samples ($N = 184$) included in CERVUS calculations except Hardy–Weinberg (H–W) calculations, which were calculated using a subset of unrelated adult individuals ($N = 33$) in GENEPOP

	Locus			
	MSMM-6	MOE-2	MSMM-2	AV-13
Allelic richness	6	12	16	21
H–W deviation (P)	0.27	0.17	0.26	0.65
Heterozygosity (H_O)	0.37	0.75	0.87	0.84
Null allele frequency	0.007	0.05	0.008	0.02
Exclusion probability (1)	0.07	0.51	0.62	0.64
Exclusion probability (2)	0.21	0.66	0.77	0.78

H_O = observed heterozygosity.

MOE-2 (frequency estimate = 0.05). However, all known offspring–maternal pairs were free of possible homozygous mismatches at this locus.

We collected 133 embryos, but some embryos were too small to yield samples uncontaminated by maternal tissues and were excluded from paternity analysis; 109 embryos provided sufficient uncontaminated tissue for microsatellite amplification. These embryos were assigned paternity with no mismatches between assigned male and embryo genotypes using CERVUS with 95% confidence interval. Of these, we excluded an additional 10 embryos for which the most likely father was less than twice as likely as the next-most likely father. Therefore we assigned paternity to 99 of 133 embryos with conservative measures of confidence. No single-pup litters were observed.

Parentage

Of the 26 litters assessed for paternity, 21 (80.8%) were sired by the paired mate of the mother. Of these 21 litters, 20 were exclusively sired by the paired male and one was sired in part by the paired male and in part by another nonpair male. Six (23.1%) litters were sired by at least one extrapair male. Two paired females (7.7%) produced mixed-sire litters. The embryos from one of these mixed-sire litters were sired by one of two extrapair males (EPF–EPF), and the embryos from the other mixed-sire litter were sired by either her paired mate or an extrapair male (IPF–EPF; Table 2).

Twenty-six of 43 (60.5%) males successfully fertilized offspring of at least one female each. Two of these 26 males (7.7%) fertilized more than one female (each siring embryos of both their respective paired female and an extrapair female; Table 2). Overall, 21 successfully breeding males (80.8%) sired embryos of their paired females, with 19 males exclusively siring litters with their pairmate. Four paired males (15.4%) and three single males (11.5%) sired embryos of a female they were not paired with for a total of seven (26.9%) male EPF litters. Thus, although a majority of paired males sired offspring with partners only, a comparable number of paired and single males fertilized young with a nonpartner (Table 2). A similar number of paired and single males did not

Table 2. Number of paired or single prairie voles and the type of fertilizations produced

	Social mating status	Single fertilizations		Multiple fertilizations		None	
		IPF	EPF	IPF/EPF	EPF/EPF		
Male	Paired	32	19	2	2	0	9
	Single	11	n/a	3	n/a	0	8
Female	Paired	31	19	3	1	1	4
	Single	5	n/a	2	n/a	0	3

IPF: intrapair fertilization; EPF: extrapair fertilization. By definition, single animals could not produce an IPF. For simplicity, data in the table are pooled across enclosures.

breed and by definition single males produced no IPFs (Fig. 2, Table 2).

We found no difference in the age, total length or weight of males or females that produced IPFs, EPFs or no fertilizations (nested ANOVAs: males: age: $F_{2,38} = 0.62$, $P = 0.55$; total length: $F_{2,38} = 1.14$, $P = 0.33$; weight: $F_{2,38} = 0.41$, $P = 0.67$; females: age: $F_{2,31} = 0.04$, $P = 0.96$; total length: $F_{2,31} = 0.36$, $P = 0.70$; weight: $F_{2,31} = 0.71$, $P = 0.50$). Similarly, we found no differences between paired and single males or females for these factors (nested ANOVAs: males: age: $F_{2,39} = 0.06$, $P = 0.94$; total length: $F_{2,39} = 0.50$, $P = 0.61$; weight: $F_{2,39} = 0.26$, $P = 0.78$; females: age: $F_{2,34} = 1.06$, $P = 0.36$; total length: $F_{2,34} = 2.30$, $P = 0.12$; weight: $F_{2,34} = 0.65$; $P = 0.53$).

Modal Mating System

Two males and one female engaged in both an IPF and an EPF and so were scored as genetically nonmonogamous (EPF) individuals. Similarly, we counted animals that engaged in multiple EPFs (one female) simply as a single EPF-mating individual.

The observed ratios of animals in each category (IPF:EPF) were 19:7 for males and 20:6 for females. The replicated G test revealed a significant departure from random mating for both males (predicted IPF:EPF = 2.9:20.1, $G = 56.74$, $P < 0.001$) and females (predicted IPF:EPF = 2.4:19.2, $G = 70.90$, $P < 0.001$). The test revealed no discernible enclosure effects for females ($G = 8.58$, $P = 0.18$), but there was significant heterogeneity in the frequency of IPF males ($G = 12.05$, $P = 0.05$). A closer examination of the G statistics for individual enclosures revealed that one enclosure,

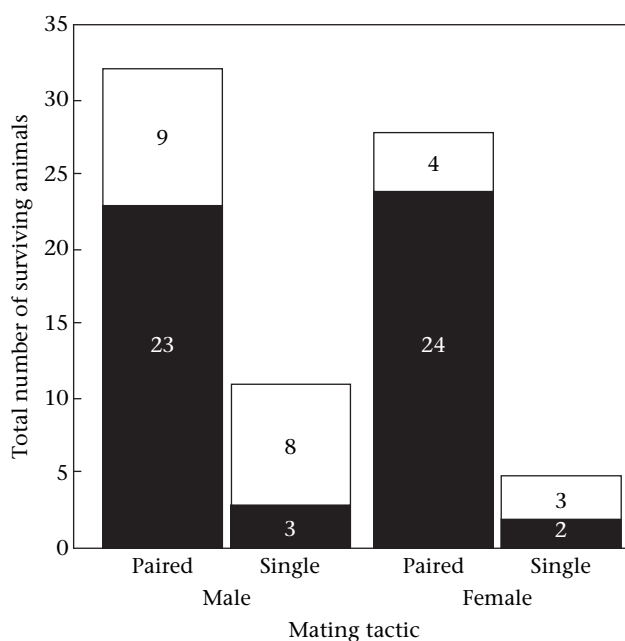


Figure 2. Number of recovered paired and single male and female prairie voles that either bred successfully (black) or did not breed (white). For clarity, data are pooled across enclosures. Analyses based on enclosure means did not differ from pooled data and are provided in text.

which had five IPF and zero EPF males, had a G statistic much higher than those of the other enclosures (observed IPF:EPF = 5:0; predicted under random mating = 0.5:3.4, $G = 23.93$, $P < 0.001$). This enclosure was run at the end of the breeding season, but the other enclosure run concurrently did not show a similar effect, suggesting that this idiosyncrasy was not related to time of year. Treating the seven enclosures separately from this one revealed the same pattern for main effects but no significant enclosure effect (male main $G = 36.9$, $P < 0.001$; heterogeneity $G = 8.14$, $P = 0.17$). Critically, we note that whereas there was heterogeneity in the frequency of IPF males, all enclosures contained more IPF males than predicted under random mating. These results were consistent with those using a simpler analysis pooling data across enclosures, which showed that prairie voles had significantly more EPFs than predicted under genetic monogamy (predicted IPF:EPF = 26:0; males: $P = 0.02$; females: $P = 0.02$; Table 3) and fewer than under random mating (predicted IPF:EPF = 7:19; males: $P < 0.01$; females: $P < 0.001$; Table 3).

Reproductive Success

For each sex, we indirectly assessed reproductive success for pairing or nonpairing by comparing the probability that an individual would reproduce, the mean number of embryos produced and the average litter size for paired and single individuals. We then assessed whether genetic monogamy provided an advantage by comparing the number of embryos sired by animals mating exclusively with their partners to animals mating singly or with a nonpair mate.

Males

Paired males were more likely to fertilize a female than were single males (mean probability of mating if paired = 0.77 ± 0.08 ; single = 0.21 ± 0.14 ; randomization test: $P = 0.003$). In a simpler analysis that pools across enclosures, a comparison of the number of successful and unsuccessful males also revealed a significant difference in these probabilities (paired males: 23 of 32; single males: three of 11; Fisher's exact test: $P = 0.01$; Fig. 2). Although paired and single males sired similar numbers of embryos when successful (randomization test: $P = 0.82$), paired

Table 3. Observed values (Obs) for male and female prairie voles that produced intrapair (IPF) and extrapair (EPF) fertilizations and expected values for genetic monogamy (Exp GM) and random mating (Exp RM)

	Males			Females		
	Obs	Exp GM	Exp RM	Obs	Exp GM	Exp RM
IPF	19	26	7	20	26	7
EPF	7	0	19	6	0	19

All $P \leq 0.05$. Random mating expectations are derived from simulations, as described under Methods. For clarity, data in the table are pooled across enclosures. A full analysis is given in the text.

males sired significantly more embryos per capita (randomization test: $P = 0.01$; Fig. 3), suggesting a fitness benefit to being paired. In contrast, males fathering one or more embryos solely with an extrapair mate had fitness comparable to those that fathered offspring solely with their partner (randomization test: $P = 0.43$; Fig. 4). Furthermore, because paired males produced more young, we compared the number of embryos sired among only paired males that either produced EPFs or IPFs and found no difference in the number of embryos they sired (randomization test: $P = 0.37$). We note that two males sired offspring both within and outside their pair; one sired eight offspring and the other sired at least three (we were unable to confidently assign paternity to six remaining embryos from two litters sired at least in part by this male).

Females

Similarly, the probability of successfully breeding for paired females (0.84 ± 0.07) was greater than that for single females (0.20 ± 0.16 ; randomization test: $P = 0.05$). Pooling the data across enclosures produced a similar trend (27 of 32 versus two of five; Fisher's exact test: $P = 0.057$; Fig. 2). Litter size did not differ significantly between successfully breeding paired or single females (randomization test: $P = 0.33$). Collectively, paired females produced more offspring than single females (randomization test: $P = 0.05$; Fig. 3). Finally, we compared the litter size of females who mated faithfully (IPF) or unfaithfully (EPF). Litter size of females fertilized exclusively by one nonpair male and those fertilized exclusively by their partners were not significantly different (randomization test: $P = 0.27$; Fig. 4). Like males, restricting the analysis to paired animals revealed no differences in the number of embryos between IPF and EPF females (randomization test: $P = 0.90$). Two females had litters with multiple sires. These litters were on the upper end of mean litter size

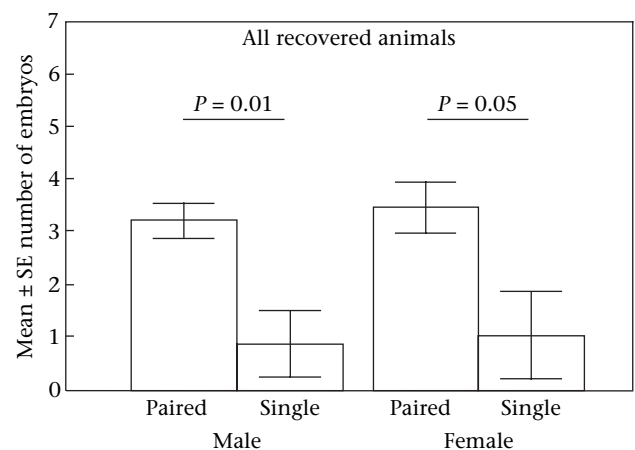


Figure 3. Mean \pm SE number of embryos from all recovered male and female prairie voles that were paired or single. Means and standard errors are based on enclosure means (paired $N_{\text{enclosures, male}} = 8$; $N_{\text{enclosures, female}} = 8$; single $N_{\text{enclosures, male}} = 8$; $N_{\text{enclosures, female}} = 5$). Total numbers of animals in each category are given in Table 2.

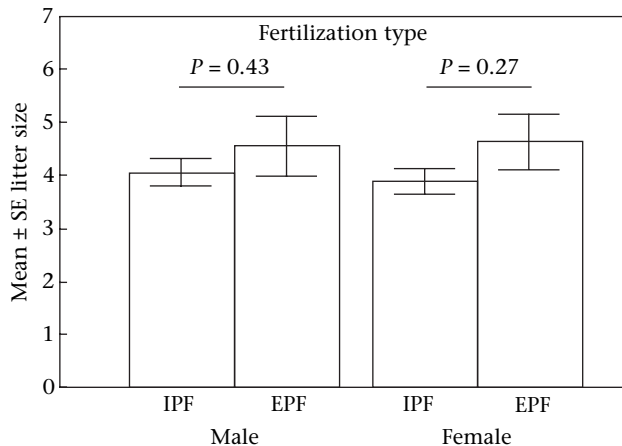


Figure 4. Mean \pm SE number of embryos from male and female prairie voles that engaged in only intrapair fertilizations (IPF; $N_{\text{enclosures, male}} = 8$; $N_{\text{enclosures, female}} = 8$) or only one extrapair fertilization (EPF; $N_{\text{enclosures, male}} = 3$; $N_{\text{enclosures, female}} = 3$). Means and standard errors are based on enclosure means. Total numbers of animals in each category are given in Table 2.

(4.4 ± 0.23) with six and seven embryos per litter. If we assume that mating multiply did not affect litter size (e.g. Wolff & Dunlap 2002) and include these two litters in our analysis as EPFs, we find that unfaithful females tend to have larger litters than females mating exclusively with their pair mate (randomization test: $P = 0.05$); however, restricting the analysis to paired females renders the effect nonsignificant (randomization test: $P = 0.26$).

DISCUSSION

Consistent with previous reports (Thomas & Birney 1979; Carter & Getz 1993; Getz et al. 1993), a majority of males and females in our study formed pairs. These patterns of space use were significant but imperfect predictors of paternity. We rejected the hypotheses that individuals mate exclusively with a partner or mate randomly among available mates. Solomon et al. (2004) observed that five of nine pregnant voles trapped in the field had multiply sired litters, which questioned the degree to which prairie voles are genetically monogamous, a point also raised by Getz & Carter (1996). Our results demonstrate that prairie voles are indeed socially but not genetically monogamous.

We found that greater breeding success for paired individuals was associated with social monogamy but not with genetic monogamy. Paired (i.e. resident) animals of both sexes had a higher probability of successfully breeding than single (i.e. wandering) animals. Thus, contrary to results of Solomon & Jacquot (2002), our results support the hypothesis that wandering males (rather than resident males) are making the best of a bad situation. Furthermore, the added fitness benefits associated with pairing (e.g. Wang & Novak 1992; Gubernick & Teferi 2000; Wolff & Macdonald 2004) were removed from our analyses; thus we probably underestimated the fitness advantages of social monogamy. To examine fitness consequences of sexual fidelity, we next focused our analysis

on those animals that bred. For both males and females, singly sired litters were of similar size regardless of pairing status or sexual fidelity. In this more narrow measure, the reproductive success of faithful and unfaithful animals was comparable. Taken together, the results suggest that the greater fertilization success of paired animals favours social monogamy; however, we cannot document benefits for sexual fidelity.

Assessing Individual Tactics

The results suggest a dissociation between social and genetic monogamy consistent with patterns in many avian taxa (Westneat et al. 1990; Birkhead & Møller 1992) but distinct from those in several other monogamous rodents (e.g. Foltz 1981; Ribble 1991, 2003; Marfori et al. 1997; Gubernick & Teferi 2000). Why should prairie voles adopt a socially monogamous mating system? Wolff & Macdonald (2004) noted that monogamy may evolve from promiscuity when males that engage in either monogamous or promiscuous matings produce comparable offspring numbers. They argue that mate guarding, paternal care and protection against infanticide may tip the balance in favour of pairing males. Our results show that the number of embryos sired by an IPF or EPF event is similar. Despite this seeming equivalence, a majority of paired males engaged in an IPF event, whereas, in contrast, a minority of both paired males and single males engaged in an EPF event (Table 2). Despite the rarity of extrapair fertilizations, males that mate with their partner and that obtain extrapair copulations may have greater reproductive success than males mating with only one female. We note anecdotally that the one male who obtained both an IPF and EPF, and for whom we were able to assign paternity to all candidate pups with confidence, sired eight offspring in total. Perhaps not surprisingly, this corresponds to roughly double the average single litter size (4.4 ± 0.23). However valuable EPFs are to males, the prevalence of mate guarding (Getz & Carter 1980) and the associated success of paired males in producing embryos suggest that IPFs dominate male fitness and EPFs are sought opportunistically.

As with males, paired females had a higher probability of successfully breeding on average and thus, by our measure, had greater reproductive success than did single females. Litter sizes of those females that did breed successfully were comparable for females who were paired and single. Litter sizes were also similar for females whose young were sired exclusively by a partner or nonpartner. Similarly, in a natural population of voles, Getz & McGuire (1993) reported that litter sizes of paired and single females were comparable. Moreover, they found that single females were shorter lived than paired females (Getz & McGuire 1993). When taken together, these findings suggest that selection favours the monogamous social mating system that we and others (e.g. Getz et al. 1993) have observed. However, when we included the two multiply sired litters among extrapair fertilizations in our analysis of both paired and single females, litter sizes from female EPFs were significantly larger than those of

faithful (IPF) females. We do not know whether multiple mating results in an increased litter size in field populations of prairie voles, but evidence from the laboratory suggests that it does not (Wolff & Dunlap 2002). What is clear, however, is that the mating success associated with sexual fidelity is no better than that associated with infidelity. Therefore, if selection favours prairie vole monogamy at all, it is probably favouring a social monogamous mating system (i.e. living arrangement) without regard to patterns of actual mating behaviour (however, see Gowaty 1996).

Although the number of single females was relatively low (five of 36), it is surprising that any females were single given that nonpairing seems maladaptive to both sexes (Bateman 1948; Kleiman 1977; Andersson 1994). In every enclosure containing a single female, at least one single male was available with which the female could have formed a pair. This observation has no clear explanation. We speculate that it may be related to natural variation in receptivity. If females vary in the degree to which they become receptive to males, then females that took longer to become receptive may not have had an opportunity to mate during the relatively brief time they were in the field (approximately 18–20 days). Alternatively, mate incompatibility or undesirability may explain why females did not form pairs. Single females may have rejected the available single males for a number of reasons including poor somatic condition, parasite load or genetic incompatibility. Considering the short life expectancy of voles (Getz et al. 1997), we suspect that lack of pairing was more likely a function of female receptivity than the availability of a suitable partner.

Obtaining a measurement of total fitness is inherently difficult and somewhat contentious (e.g. Arnold & Wade 1984; Byerly & Michod 1991). Inferences of fitness include counts of mating events, adult survivorship and quantifying the production of offspring (e.g. see Arnold & Wade 1984; Clutton-Brock 1988). Although incomplete, focusing on a single component of fitness can provide a clearer view of exactly how particular phenotypes contribute to fitness. By focusing on fertilization success (an important component of fitness), we separated reproductive success through mate guarding or female choice from postpartum effects on pup survival (e.g. parental care). Whereas not all embryos would have survived to reproductive maturity (Getz et al. 1979), the quantification of embryos represents an upper limit on an individual's potential fitness per litter. Moreover, because biparental care appears to enhance pup survival (Wang & Novak 1992; Gubernick & Teferi 2000; Wolff & Macdonald 2004), we are probably underestimating the value of pairing.

Measuring fertilization success provides a proximal assessment of fitness; additional measures of fitness (e.g. individual survivorship or number of mates) will be needed to evaluate total fitness before a complete evaluation of the costs and benefits of social or genetic monogamy can be provided. Ultimately it will be necessary to investigate the consequences of the variation between mating tactics to be sure that the cross-sectional measure of reproductive success that we used translates into later measures representative of total fitness.

The Prairie Vole as Mammalian Model

Although prairie voles have become an icon of monogamous behaviour, several prior studies suggest that their pair bonding may not translate into sexual fidelity. In the field, a significant minority of males and females live singly (Getz & Hofmann 1986). Reproductive single females were thought to be widows, and whether single males reproduced was unknown because parentage analysis was not performed (Getz et al. 1993). Solomon et al. (2004) sampled nine pregnant females and found multiple paternity within several litters. Although this indicated multiple mating, the current and past pairing status of these females was unknown. We demonstrate that both single males and females reproduce, even if having never paired. Indeed, the short duration of our study and laboratory evidence of mate switching (Wolff et al. 2002) suggest that we have underestimated the number of lifetime sexual partners; the fitness consequences of life-long attachment or mate switching remain to be tested.

The existence of substantial extrapair mating in our data contrasts with findings from other New World monogamous rodents (Foltz 1981; Ribble 1991, 2003; Marfori et al. 1997; however, see Goossens et al. 1998) and caution against simple extrapolations from one model system to distantly related species. A more systematic documentation of species differences in behaviour and neurobiology promise a more complete understanding of social attachment and its variations (e.g. Insel & Shapiro 1992; Bester-Meredith et al. 1999; Insel & Young 2001; Ribble 2003; Fink et al. 2006). Somewhat ironically, this distinction between prairie voles and other monogamous rodents, the dissociation of social and sexual fidelity, leads us to suggest that prairie voles are even better models of human attachment than has been appreciated.

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