Length polymorphism at the *avpr1a* locus is correlated with male reproductive behavior in a natural population of prairie voles (*Microtus ochrogaster*)

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Abstract Laboratory studies have shown that vasopressin can influence sociosexual behavior through its action on the vasopressin 1a receptor (V1aR). There is substantial evidence that the length of a microsatellite in the gene (*avpr1a*) encoding for the V1aR can affect social attachment to females and paternal behavior in male prairie voles under laboratory conditions. However, previous field studies of prairie voles have failed to detect a strong effect of the length

of a male's *avpr1a* allele on their sociosexual behavior but these studies are typically much shorter than the average prairie vole breeding lifespan. We examined the relationship between male *avpr1a* microsatellite allele length and sociosexual behavior in a natural population of prairie voles for 15 weeks, closer to the lifespan of prairie voles in nature. Contrary to predictions, we found that males with the longest *avpr1a* microsatellite alleles were significantly more likely to sire offspring with more than one female and to sire offspring that survived until trappable age than males with the shortest *avpr1a* microsatellite allele lengths. This relationship was the strongest for males with the longest tenure on the study site. As in previous field studies, we did not find evidence of a relationship between a male's *avpr1a* genotype and any index of social behavior including male residency status or the number of females with which males associate. This is the first study to support the hypothesis that a male's *avpr1a* genotype is a factor underlying variation in the genetic mating system of prairie voles under natural conditions.

Keywords *Avpr1a* • Prairie vole • Reproductive success • Sociosexual behavior • Vasopressin

Introduction

In many species of animals, individuals within as well as between geographically distinct populations exhibit intraspecific variation in behaviors associated with reproduction and parental care (e.g., Gross 1984, 1996; Brockmann 2008; Taborsky 2008; Wolff 2008; Schradin et al. 2012). Since variability in reproductive behavior can ultimately affect an individual's lifetime reproductive success (Mock and Fujioka 1990; Gross 1996; Gubernick and Teferi 2000; Schradin and Lindholm 2011), determining the mechanisms underlying this variation is crucial for understanding the evolution of mating systems and social behavior. Empirical data from numerous field studies suggest that intraspecific variation in reproductive behavior is frequently an adaptive response to differences in environmental conditions (e.g., density; habitat structure; Penteriani et al. 2011; Schradin et al. 2012) or individual characteristics (e.g., body size, age, developmental stage, Young et al. 2007; Oliveira et al. 2008). More recently, an increasing number of laboratory studies have provided an abundance of information on neurobiological mechanisms that mediate sociosexual behavior both within as well as among species (Insel 1997; Lim et al. 2004; Donaldson et al. 2010; McGraw and Young 2010). These studies suggest that the basis for intraspecific variation in sociosexual behavior appears to be complex, perhaps involving multiple ecological and neurogenetic influences.

Several neuropeptides can influence the expression of sociosexual behaviors in diverse animal taxa (Witt 1995; Insel and Young 2000; Heinrichs and Domes 2008; Goodson and Thompson 2010; Oldfield and Hofmann 2011; Godwin and Thompson 2012). In mammals, behaviors such as social attachment, partner preference, and paternal care are mediated by the neuropeptide vasopressin through its action on the vasopressin 1a receptor (V1aR; Hammock and Young 2002; Hammock et al. 2005; Donaldson et al. 2010). Several lines of evidence

indicate that differences in the neural expression of V1aR contribute to inter- and intraspecific variation in mammalian sociosexual behavior. Among arvicoline rodents, the socially monogamous prairie voles (*Microtus ochrogaster*) and woodland voles (*M. pinetorum*) share similar neural patterns of V1aR distribution and densities that differ significantly from those in the nonmonogamous meadow (*M. pennsylvanicus*) and montane (*M. montanus*) voles (Hammock and Young 2002; but see Fink et al. 2006). Male transgenic house mice (*Mus musculus*) and meadow voles possessing the *avpr1a* gene from prairie voles have a neural distribution of V1aRs similar to that in prairie voles and exhibit increased partner preference and affiliative behavior towards females relative to nontransgenic males (Young et al. 1999; Lim et al. 2004). V1aR signaling is necessary for the formation and expression of female partner preference in experimentally manipulated male prairie voles. Experiments utilizing a viral vector to increase the density of the V1aR in the ventral pallidum of male prairie voles increased partner preference relative to control males (Pitkow et al. 2001). The opposite was found when RNA interference was used to decrease expression of V1aR in the ventral pallidum of male prairie voles (Barrett et al. 2013). These and other studies suggest that differences in the neural distribution and density of V1aR can lead to variation in behaviors such as the formation of social attachments and parental behavior in some mammals.

Although prairie voles are considered to be socially monogamous based on extensive laboratory and field data (e.g., Carter et al. 1986; Getz et al. 1993), variation in social and genetic monogamy occurs within all natural populations studied (Illinois: Getz et al. 1993; Kansas and Indiana: Mabry et al. 2011; Streatfeild et al. 2011) as well as within populations maintained in semi-natural enclosures (Ophir et al. 2008a; Solomon et al. 2009). In natural and semi-natural populations, most male prairie voles are territorial (resident males) and reside at a nest with one

female but a sizeable minority (32 – 46 %) are nonterritorial (wandering males) with home ranges that overlap multiple females (Getz et al. 1993; Solomon and Jacquot 2002; Ophir et al. 2008a). The degree of social monogamy varies within and between populations over time (Fitch 1957; Roberts et al. 1998; Cushing and Kramer 2005; Streatfeild et al. 2011). In addition, in natural and semi-natural populations some males sire litters with multiple females while other males sire offspring with just a single female (Ophir et al. 2008a; Ganev et al. 2009; Solomon et al. 2009; Mabry et al. 2011). Multiple paternity has also been reported in litters from females in natural (Illinois: Solomon et al. 2004; Indiana and Kansas: Mabry et al. 2011) and semi-natural populations (Ophir et al. 2008a; Ganev et al. 2009; Solomon et al. 2009).

Some of the inter- and intrapopulational variation in sociosexual behavior observed in prairie voles appears to be due to ecological factors such as population density and the distribution of vegetation (Streatfeild et al. 2011) or differences in individual condition such as body mass and parasite load (Chesh et al. 2012). There is also evidence that suggests that individual differences in the pattern of V1aR neural expression may explain some of the variability in reproductive behavior within or among populations. In prairie voles, polymorphism in the length of the microsatellite DNA within the regulatory region of the gene (*avpr1a*) encoding for V1aR predicts individual differences among males in the expression of V1aRs in specific areas of the brain that appear to influence behaviors such as partner preference and paternal care (Hammock and Young 2002, 2005; Hammock et al. 2005). Interestingly, there were some inconsistencies among these studies with respect to which brain areas showed differential V1aR expression, suggesting factors other than microsatellite length influence expression. The differences in neural expression of V1aR in some brain regions are correlated with some male sociosexual behavior under laboratory conditions (Pitkow et al. 2001; Hammock

and Young 2005). Specifically, in laboratory trials, males with longer *avpr1a* microsatellite alleles exhibited greater expression of some behaviors characteristic of social monogamy (partner preference, paternal care, affiliation) relative to males with shorter *avpr1a* alleles (Hammock and Young 2005). These data suggest that a male's *avpr1a* genotype influences his neural expression of V1aRs, which in turn affects his social behavior and provide a neurogenetic mechanism for explaining some of the variation in male sociosexual behavior observed in natural populations.

However, attempts to relate variation in male sociosexual behavior in field populations to polymorphism in the length of a male's *avpr1a* microsatellite alleles have failed to show strong support for this hypothesis. No significant relationships were detected between male *avpr1a* genotype and indices of social and genetic monogamy from 4-8 week studies of natural populations of prairie voles (Mabry et al. 2011). There was also no evidence that the *avpr1a* genotype of male prairie voles predicted variation in several indices of social monogamy such as space use or the likelihood of a male adopting a resident versus wanderer (male found at >1 nest) tactic when semi-natural populations were studied for 3-weeks by Ophir et al. (2008b) or 15-weeks by Solomon et al. (2009). In the Solomon et al. (2009) study, genetic parentage data indicated that males with shorter *avpr1a* microsatellite alleles sired significantly more offspring with more females relative to males with longer *avpr1a* microsatellite alleles, but no such relationship was detected in the shorter-term Ophir et al. (2008b) study. Since laboratory settings are much less complex than the field environment, it is not unusual to find that results from a laboratory setting do not translate to the field (Calisi and Bentley 2009).

Recent work by Donaldson and Young (2013) provides direct evidence that the prairie vole *avpr1a* microsatellite region influences neural V1aR expression in specific brain regions but

they also report that other genetic polymorphisms mediate neural V1aR expression and this may be contributing to inconsistencies among studies. It has also been suggested that no simple relationship exists between *avpr1a* allele length and behavior because length may be a rough indicator of sequence differences that are the basis of the V1aR expression variation (Phelps et al. 2010). Another reason for the inconsistencies in the studies in semi-natural and free-ranging populations with respect to the relationship between male *avp1a* genotype and reproductive success may be the differences in length of the studies. The minimum interbirth interval among female prairie voles is about three weeks (Keller 1985) and in the two shorter studies (Ophir et al. 2008b; Mabry et al. 2011) almost all females had just a single litter while in the study by Solomon et al. (2009) females had up to four litters. Since the lifespan of prairie voles averages 30-122 days depending on factors such as density, season of birth and dispersal (Getz et al. 1994), a longer time interval may need to be studied to more accurately assess the relationship between *avpr1a* polymorphism and sociosexual behavior in free ranging populations, particularly if the *avpr1a* microsatellite only has a modest influence on neural V1aR expression as suggested by Donaldson and Young (2013).

Therefore, the objective of this study is to examine the relationship between male *avpr1a* genotype and sociosexual behavior in a free ranging population of prairie voles over a period of time similar to the average adult lifespan to test the hypothesis that *avpr1a* length polymorphism is contributing to the variation in male social attachment and mate fidelity observed in free-living populations. If microsatellite length polymorphism at the *avpr1a* locus is an important factor contributing to individual differences in sociosexual behavior in nature, we expected to detect relationships between male *avpr1a* genotype and some indices of social and reproductive behavior measured in the field. Specifically, as suggested by the partner preference study by

155 Hammock and Young (2005) and supported by the results of our study in semi-natural
156 populations (Solomon et al. 2009), we expected that males with longer *avpr1a* microsatellite
157 alleles would have a higher residency score (i.e., be classified as residents more often than as
158 wanderers), associate with a smaller proportion of females, show a greater amount of home range
159 overlap with the female that the male overlapped with most, associate with her the most, and sire
160 offspring with fewer females relative to males with shorter *avpr1a* microsatellite alleles. If the
161 number of females that a male sires offspring with is closely associated with his reproductive
162 success then males with longer *avpr1a* microsatellite alleles should have lower reproductive
163 success (i.e., sire fewer offspring) than males with shorter *avpr1a* microsatellite alleles.
164

165 **Materials and methods**

166 Study site and animals

167 The study site was located approximately 5 km north of Bloomington, Indiana, within the
168 Indiana University Bayles Road Preserve (39°13'00"N, 86°32'27"W). The site is an old field,
169 primarily composed of grasses and forbs and is maintained by periodic mowing to prevent
170 successional changes. Data were collected from a 10,000 m^2 (1 ha) area during a 15-week period
171 from early-June to mid-September 2010, coinciding with the mid- to late-breeding season of
172 prairie voles (Getz et al. 1993). We monitored prairie voles within the study area by live-
173 trapping using Ugglan multiple-capture traps (Grahnab, Hillerstorp, Sweden). Traps were baited
174 with cracked corn, a low quality food item (Desy and Batzli 1989; Cochran and Solomon 2000)
175 and shielded from heat and rain by aluminum flashing covered with vegetation. Every time an
176 individual was captured, we recorded the following: location of capture, identification number,

177 sex, body mass, age class, reproductive status (males: scrotal or non-scrotal, females: pregnant
178 [determined by gently palpating a female's abdomen], lactating, or non-reproductive), body mass
179 and any individuals with which they were captured. Upon first capture, all individuals were
180 given a unique toe clip for identification and these tissue samples were stored at -20 °C for future
181 genetic analysis. Body mass was determined to the nearest gram using a Pesola micro-line
182 spring scale (Forestry Suppliers Inc., Jackson, MS, USA). Animals >29 g in mass were
183 classified as adults, while animals 21-29 g were considered subadults, and animals <21 g were
184 classified as juveniles (Gaines et al. 1979; Getz et al. 1993).
185 During the first three weeks of the study, we trapped on a 21x6 grid with grid points
186 approximately 10 m apart and one trap per grid point. During each grid-trapping week, we
187 checked the traps eight times. Traps were open from 2000 on Sunday until 0700 on Monday
188 when they were checked and left unset. Traps were reset at 1800 on Monday, Tuesday,
189 Wednesday, and Thursday nights with trap checks taking place on those nights at 2000 as well as
190 0700 the next mornings with the exception of Wednesday (i.e., Tuesday, Thursday and Friday
191 mornings). Traps were set from 1800 to 2000 and overnight until 0700 (except Wednesdays) to
192 avoid heat related trauma to trapped animals. At all other times, traps were left in place but
193 unset. Grid trapping allowed us to monitor population density, estimate home range size and
194 space use, and capture females for radio-tracking to their respective nests.
195 We radio collared all adult females caught during the first 3 weeks of grid trapping and
196 used radio-telemetry to locate their nests. Females to be radio tracked were fitted with model
197 PD-2C radio transmitters (Holohil Systems Ltd, Ontario, CA) attached around the neck of the
198 individual with a small zip tie covered in rubber tubing (see Keane et al. 2007; Lucia et al. 2008
199 for details). The radio collars weighed approximately 3 g and were always less than 10% of a

female's body mass. A radio collar of less than 10 % of a female's body mass should not significantly influence her daily energy expenditure (Berteaux et al. 1996). Furthermore, Pouliquen et al. (1990) found no negative effects on social interactions of wild mice, *Mus domesticus*, after attachment of radio collars. Radio-tracking was conducted via triangulation using two FieldMaster receivers (Johnson's Telemetry, Ed Dorado Springs, MO) and three-element Yagi antennas (Johnson's Telemetry, Ed Dorado Springs, MO). Fixes were taken for each female twice a day, once between 0900 and 1000 and again between 1600 and 1700, because it was assumed that females would likely be underground at these times. Nest locations were determined by searching for surface nests or entrances to underground nests in the vicinity where females were tracked. A female had to be tracked to the same nest location for a minimum of 3 consecutive days to confirm that she was living at that nest location. Once a nest location was confirmed, we placed three Ugglan traps in the surface runways within 1 m of nest entrances and the female's radio collar was removed during her next capture. If a female's nest was not determined by the end of the 5^{th} week, the collar was removed upon the female's next capture.

After the third week of grid trapping, we switched to nest trapping for two weeks, followed by one week of grid trapping. During the last 12 weeks of the study, we divided the trapping into 4 periods, with each period composed of two nest trapping weeks and the following grid trapping week. During nest trapping weeks, only the traps at the female nests were checked. We checked nest traps 10 times during each nest trapping week. Nest trapping followed the same schedule as grid trapping but we also set traps at 1800 on Sundays and Fridays followed by checks at 2000 on those days. As with grid trapping, traps were left in place but unset at all other

222 times. Nest trapping allowed us to determine which individuals were residents at nests, which
223 adult males associated with each adult female, and to capture juveniles for parentage analysis.
224 Adult prairie voles may change residency status (resident versus wanderer) or social
225 associations with females during their lifetime (Solomon and Jacquot 2002; McGuire and Getz
226 2010). Therefore, we determined adult male space use, residency status, and social associations
227 with adult females separately for each of the 4 trapping periods during the final 12 weeks of the
228 study.

229

230 Space use

231 Traditional estimators of home range size were not useful for this study because many voles
232 were captured only a few times. The number of captures per individual ranged from 1 to 109,
233 with 47.5% of voles being captured ≤5 times. When individuals are trapped only a few times,
234 the mean square distance (MSD) moved by individuals from their center of activity can be a
235 suitable method to estimate home range size, because only 3 different capture locations are
236 needed to calculate the geometric center of a two-dimensional region (e.g., home range, Slade
237 and Russell 1998). Slade and Russell (1998) showed that the MSD moved by prairie voles from
238 their center of activity was more highly correlated with other estimates of home range size, such
239 as minimum convex polygon, harmonic mean or kernel densities, than other measurements of
240 distance moved. Therefore, we estimated adult male and female prairie vole home range size by
241 calculating the MSD moved from an individual's center of activity (Slade and Swihart 1983;
242 Diffendorfer et al. 1995; Streatfeild et al. 2011). A vole's center of activity was calculated as the
243 mean X and Y coordinates of all an individual's captures from grid trapping and represents the

geometric center of an individual's grid capture locations (Hayne 1949). For each of the four 3-week periods of trapping data, we calculated the MSD of all adults trapped at ≥3 different locations during the grid trapping week of a particular trapping period. Home range sizes were not calculated for the fourth time period, due to the presence of a raccoon on the field site that disturbed many traps during the last week of grid trapping.

The number of females with which a male's home range overlapped was quantified from grid trapping data for each 3-week trapping period. In addition, for each trapping period we calculated the percentage overlap of a male's home range with the home range of the female that the male's home range overlapped the most. A male and female were considered to overlap (share a trap location) if each were captured at least once during the same grid-trapping week at the same trap location but not necessarily the same time or day. To determine the proportion of overlap with the most overlapped female, the number of shared grid trap locations with the female with which the male shared the most trap locations was divided by the total number of locations at which the male was trapped (Streatfeild et al. 2011).

Social associations and nest residency

To evaluate the number of female social ties per male, we determined the number of unique females with which each male associated during each 3-week trapping period and the pairwise half-weight association index (AI; Cairns and Schwager 1987; Whitehead 1999, 2009; Mabry et al. 2011) between every male and each female with which he associated during each period. The AIs were calculated using SOCPROG software and customized coding in R. A male was defined as "associating" with a female when both were captured at the same nest, but not

266 necessarily in the same trap, during the same trap check. We also determined a male's relative
267 association index (relative AI) with the female with which he associated most, to quantify the
268 relative strength of each male's social interactions with the female(s) with which he associated.
269 Relative association index was calculated by dividing a male's AI with the 'most associated
270 female' by the sum of the AIs with all females with which he associated during a given trapping
271 period (Mabry et al. 2011). Relative AI is conceptually very similar to the relative encounter rate
272 used by Ophir et al. (2008a) to quantify the relative overlap of male and female prairie vole
273 home ranges. A relative AI of ≥0.5 indicates that a male associated with a given female more
274 often than with all other females combined and a relative AI value of 1 indicates that a male
275 associated with only one female.
276 Data from nest trapping weeks was used to determine the residency status of sub-adult
277 and adult males. Since males may change their residency status during their lifetime (Solomon
278 et al. 2009; McGuire and Getz 2010), we determined the residency status of males for each 3-
279 week trapping period. Sub-adult and adult males were classified as a resident at a particular nest
280 if ≥75% of their captures were at one nest during a particular 3-week trapping period and they
281 were captured at least once during each nest trapping week during that period (Cochran and
282 Solomon 2000). Conversely, sub-adult and adult males that did not have ≥75% of their captures
283 at a single nest site but were captured at least once during each nest trapping week during a 3-
284 week time period were classified as wanderers. Males not captured at least once during each
285 nest-trapping week within a particular 3-week trapping period were considered likely to be
286 visitors that lived outside the trapping grid and were not included in any analyses. For each
287 trapping period, residents were given a residency score of one while wanderers received a score
288 of zero. A male's average residency score over the four trapping periods was used as an index of

the extent to which a male adopted a resident versus a wanderer strategy (Solomon et al. 2009). Trapping periods when a male could not be classified as a resident or wanderer were excluded from the calculation of a male's residency score.

Avpr1a microsatellite analysis

We determined the *avpr1a* genotypes of males using the polymerase chain reaction (PCR) to amplify the *avpr1a* microsatellite region of the V1aR using primers designed specifically for prairie voles (Hammock and Young 2005; Solomon et al. 2009; Mabry et al. 2011). Genomic DNA for microsatellite analysis was extracted from toe clips using DNeasy extraction kits (Qiagen, Valencia, CA, USA). PCR amplification was conducted in 15 µl reactions containing 100 ng/µl DNA, 10 mM Tris-HCL, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.67 µM of the forward (fluorescently labeled with 6-FAM phosporamidite; IDT DNA Technologies, Coralville, IA, USA) and reverse primers and 0.5 U of GoTaq DNA polymerase in the supplied reaction buffer (Promega Corp, Madison, WI, USA). After denaturation at 94 °C for 2 min, PCR reactions were cycled 25 times, with denaturation at 94 °C for 15 s, annealing for 20 s at 52 °C and extension at 72 °C for 45 s followed by a final elongation for 7 min at 72 °C. PCR products were diluted and combined with an internal size standard (ROX GS2500, Applied Biosystems, Foster City, CA, USA) and fragments detected using an ABI 3730 DNA sequencer with 50 cm capillary length (Applied Biosystems, Foster City, CA, USA). Base pair (bp) lengths of PCR products were determined using Genemapper (version 3.7) fragment analysis software (Applied Biosystems, Foster City, CA, USA). The *avpr1a* microsatellite region consists of multiple types of repeat units that makes binning the fragments into discrete allele size categories difficult. Therefore,

we quantified the microsatellite allele lengths using the raw bp measurements from GeneMapper (version 3.7) and treated *avpr1a* allele length as a continuous variable (Mabry et al. 2011).

Parentage analysis

Genomic DNA used for analyzing male *avpr1a* genotypes was also used for parentage analysis. To assess parentage, all live-trapped voles were genotyped at six microsatellite loci shown to be polymorphic in prairie voles using PCR to amplify microsatellite DNA (for details of PCR reactions see Keane et al. 2007; Solomon et al. 2009). PCR products were diluted and combined with an internal size standard (LIZ GS500, Applied Biosystems, Foster City, CA) and fragments were detected using an ABI 3730 DNA sequencer with 50 cm capillary length (Applied Biosystems, Foster City, CA, USA). Base-pair lengths of the fluorescently labeled DNA fragments were analyzed using Genemapper (version 3.7) fragment analysis software (Applied Biosystems, Foster City, CA, USA) and microsatellite alleles compiled into discreet size classes using FlexiBin (Amos et al. 2006).

We used the program Cervus 3.0 to calculate the polymorphic information content, and observed and expected heterozygosity at each locus used in the study. For each locus, Cervus tests if genotypic distributions deviated from Hardy–Weinberg expectations using chi-square goodness of fit tests (Kalinowski et al. 2007). Cervus 3.0 was also used to conduct a genetic determination of parentage for juveniles trapped in the field (Kalinowski et al. 2007). The Cervus 3.0 software calculates a likelihood score for each candidate parent to identify the male and female that was most likely to be the biological parents of a specific offspring. The statistical confidence of these parentage assignments is calculated using a simulation that takes

into account population allele frequencies, an estimate of genotyping error, proportion of missing genotypes, total number of candidate parents sampled, and the proportion of candidate parents sampled. The simulation was performed for 10,000 cycles with a genotyping error rate of 0.02. This error rate was based on empirical estimates of two potential sources of error: mutation and mis-scoring of alleles (Solomon et al. 2004). The remaining input parameters for the simulation were based on the actual data from the study population.

For each juvenile trapped in the field, only individuals classified as adults or older sub-adults (25-29 g) at least five days prior to the date a particular juvenile was estimated to have been conceived were considered candidate parents for parentage analysis. The birth dates of individuals first trapped as juveniles were estimated based on their body mass from live-trapping data. Pups typically weighed 2-3 g at birth and gain approximately 0.6-1.0 g daily (unpubl. data) until weaning (21 d). The conception date was then estimated by subtracting 21 d from the estimated birth date since 21 d is the average length of gestation for prairie voles (Richmond and Conway 1969; Nadeau 1985). We used this method to estimate the date of conception for each juvenile to determine candidate parents for parentage analysis. For each juvenile, we only considered candidate parents trapped within 20 m of the juvenile's site of first capture (either a natal nest or grid trap) as possible biological parents (see Winters and Waser 2003; Mabry et al. 2011). A distance of 20 m was used in the analysis because it is the approximate average home range diameter of adult prairie voles in this population (Streatfeild et al. 2011).

Parentage was assigned using the parent-pair analysis option in Cervus 3.0 and a male and female were accepted as the parents of a particular juvenile only if Cervus assigned both parents with a confidence level of ≥95 %. If after the initial analysis, a mother but not a father was assigned as a parent with at least 95 % confidence, we reran the analysis using the 'known

356 mother' option in Cervus 3.0, again only considering adult males captured within 20 m of the
357 juvenile's site of origin as candidate fathers. We used the genetic parentage data to determine
358 the total number of offspring sired by each male and the number of female partners with which a
359 male sired offspring. We could only determine how many different females a male successfully
360 sired young with, not necessarily all of the females with which a male mated.

361

362 Ethical note

363 All procedures involving the trapping, marking and handling of prairie voles were
364 approved by the animal care and use committees of Miami University and Indiana University,
365 and were consistent with the guidelines published by the American Society of Mammalogists
366 (ASM; Sikes et al. 2011) for the use of wild animals in research.

367 We individually marked prairie voles in the field using uniquely-coded combinations of
368 toe clips. Although more benign marking methods are available for marking rodents, we believe
369 that none of them were as suitable for permanently marking field populations of prairie voles as
370 toe-clipping. Non-toxic dyes and hair clipping can be used to mark mammals but these
371 identifying marks are temporary because the fur is shed or re-grows (Johnson 2001; Sikes et al.
372 2011). Since we studied the field population for almost four months, we needed to have a means
373 of marking the animals with a more permanent identification mark. Also, these techniques are
374 not appropriate for marking altricial neonates that are hairless. More permanent marking
375 methods for field populations of mammals recommended by the ASM (Sikes et al. 2011) include
376 freeze branding, ear-notching, tattooing, ear tagging, passive integrated transponders (PIT tags)
377 and toe-clipping. Each of these procedures probably causes some amount of stress or pain to the

animal. Freeze-brands, ear notches and tattoos were inappropriate for this study because of the limited number of unique marks that can made, particularly on newly caught neonates. Adult prairie voles have very small external ear pinnae and ear tag loss in prairie voles has been estimated to be 10-16% (Wood and Slade 1990; Harper and Batzli 1996) when the tags were lost by being ripped from the ears (e.g., when caught on vegetation) of tagged individuals. In addition to causing pain, the loss of ear tags could compromise the intergity of our trapping data since individuals cannot be positively identified after the loss of their tags. Finally, Wood and Slade (1990) found no detrimental effects of toe-clipping on prairie vole survival and body mass relative to ear tagging. Recent advances in technology have made it possible to mark animals for individual identification using PIT tags implanted subcutaneously. We believe this method has several drawbacks for marking prairie voles relative to toe-clipping. First, the small size of juveniles may preclude the use of PIT tags. There is no published information that indicates how large an animal must be to safely inject a PIT tag. Injecting a PIT tag into a juvenile prairie vole may be risky due to the invasive nature of the procedure and the gauge of the needle needed to inject the tag. Second, the invasive nature of the procedure also leaves implant wounds that can become infected in voles (Harper and Batzli 1996). A study by Harper and Batzli (1996) also found that 5% of the voles PIT tagged lost their tag which could compromise our ability to track specific individuls trough time.

The ASM permits the use of toe-clipping to mark animals when none of their other recommended marking methods appear suitable, especially if tissue samples also need to be collected (Sikes et al. 2011). We felt that toe-clipping was the most feasible method of permanently marking the large numbers of prairie voles of varying size classes in our study and it also allowed us to collect tissue samples for parenatge analysis. We used a clean, sharp pair of

scissors to remove toes and no more than 1 toe per foot was clipped. The ASM guidelines also do not recommend the use of anesthetics or analgesics during toe-clipping because of the prolonged period of restraint that is necessary to apply them, and because consumption of analgesic substances by licking may cause additional stress to the animal (Sikes et al. 2011). Toes were stored in 1.5 ml microcentrifuge tubes at -20 °C until DNA was extracted for parentage analysis.

Statistical analysis of data

Previous studies have demonstrated that analyses of the effect of male *avpr1a* microsatellite allele length on social and reproductive behavior yield similar results when using the summed length of a male's two *avpr1a* microsatellite alleles or either just the longer or shorter of a male's two *avpr1a* microsatellite alleles as the independent variable (Hammock et al. 2005; Ophir et al. 2008b; Solomon et al. 2009; Mabry et al. 2011). Therefore, we examined the correlation between male *avpr1a* genotype and all indices of social and reproductive behavior using the sum of a male's two *avpr1a* microsatellite lengths as the metric of a male's *avpr1a* genotype (additive genetic model). We determined if the distribution of summed *avpr1a* microsatellite allele lengths among males differed from a normal distribution with a Shapiro-Wilk test.

We analyzed the indices of space use and social associations during each of the four 3-week trapping periods separately, so that we could monitor changes in an individual male's behavior (e.g., residency status, home range size, overlap with females and association indices), throughout the last 12 weeks of the study. The relationship between male *avpr1a* microsatellite length and the mean square distance moved by males or the proportion of females in the

population overlapped by males was analyzed using linear regression with male *avpr1a* microsatellite length as the independent variable. Due to the uneven distribution of the response variable, the proportion of home range overlap between males and the adult female each male overlapped with the most was examined using logistic regression, with male *avpr1a* microsatellite length as the independent variable. A value of 1 was assigned to males whose proportional overlap with their most overlapped female was ≥0.75, while male's whose proportional overlap with their most overlapped female was <0.75 were assigned a value of zero. A value of 0.75 was used for categorizing a male's overlap with his most overlapped female since males and females that had 75 % of their captures at the same nest site were considered to be co-residents.

The relationships of male *avpr1a* microsatellite length with the number of females with which a male associated (AI) and the relative strength of a male's association with the most associated female (relative AI) were examined using logistic regression with male *avpr1a* microsatellite length as the independent variable. We choose to use a logistic regression model because there were not enough different response values to make a continuous-type analysis reasonable since most males associated with 1 or 2 females. If a male associated with only 1 female, an AI value of 0 was assigned, whereas if a male associated with more than 1 female an AI value of 1 was assigned for analysis. Relative AI values <1 were given a value of 0 and relative AI values of 1 remained at a value of 1 for analysis. These values were assigned for both AI and relative AI to compare socially monogamous males (associating with only one female) and non-socially monogamous males (associating with >1 female) against their respective *avrp1a* genotype. Additionally, the relationship of male *avpr1a* microsatellite length with a male's average residency score over the four trapping periods was examined using logistic

446 regression, where a value of 0 was assigned to all voles with an average score <1 (wanderers)
447 and a value of 1 assigned to all voles with an average score equal to 1 (residents).

448 While measures of most social indices were evaluated separately during each of the four
449 3-week trapping periods in order to account for changes in social behavior through a male's
450 lifespan, the number of offspring sired per male and number of females with which a male sired
451 offspring were calculated over the entire length of the study to estimate lifetime reproductive
452 success and genetic monogamy. The relationship between *avpr1a* microsatellite length and
453 whether or not a male sired any offspring were conducted using logistic regression. Males that
454 sired any offspring were assigned a value of 1, whereas a value of 0 was assigned to males that
455 did not sire any offspring.

456 The number of offspring sired per male and the number of females with which a male
457 sired offspring were ordinal values that fell within a narrow range. Therefore, the relationship
458 between male *avpr1a* microsatellite lengths with these two dependent variables was analyzed
459 using ordinal regression, since the number of offspring and female partners can easily be
460 partitioned into several ordinal categories. If a male did not sire any offspring, a value of 0 was
461 assigned. A value of 1 was assigned to males that sired 1 offspring and a value of 2 was
462 assigned to males that sired >1 offspring. For the ordinal regression that examined the
463 relationship between male *avpr1a* microsatellite length and the number of reproductive partners,
464 males that did not have any reproductive partners were assigned a value of 0, a value of 1 was
465 assigned to males with 1 reproductive partner and a value of 2 was assigned to males with >1
466 reproductive partner. In addition, the length of time adult males were known to be in the study
467 population (an index of survival) was included as a covariate in the ordinal regression because
468 males that were present on the study grid for just a few weeks likely sired fewer offspring with

fewer females irrespective of male *avpr1a* genotype relative to males that were present most of the study. The length of time adult males were known to be in the study population (hereafter referred to as tenure) was measured as the number of days between a male's first and last capture.

The ordinal regression model used here is based upon the cumulative logit function. That is, it models a series of log odds by way of a linear combination of predictors. More specifically, and simplified to include just a single continuous predictor for clarity,

$$\text{logit } P(Y \leq j) = \log\left[\frac{P(Y \leq j)}{1 - P(Y \leq j)}\right] = \alpha_j + \beta_1 X_1$$

where Y is the response (e.g., number of partners), j represents response category j (e.g. 0, 1, or more than 1 partner), α_j is an intercept unique to response category j, and X_1 is some continuous predictor. Inherent in this model is the assumption that the same regression coefficients (β_1 in the example above) can be used to represent the relationship between the predictor and the logit, for any response category j. Therefore, in the so-called proportional odds model, there is a single set of coefficients in the model (Hilbe 2009). The proportional odds model was fit using the VGAM package in R (Yee 2010). We tested this assumption for the models presented in this paper using a likelihood ratio test (Yee 2010), and found that the proportional odds assumption was appropriate.

In order to use an ordinal regression to analyze the number of female mates and number of offspring against summed *avpr1a* microsatellite length, both male *avpr1a* microsatellite length and tenure were categorized into quartiles (*avpr1a* [bp length]: Q1 = 1390.34-1456.59, Q2 = 1456.6-1469.45, Q3 = 1469. 64-1481.29, Q4 = 1481.3-1523.22; tenure [d]: Q1 = 1-21, Q2 = 22-40, Q3 = 41-73, Q4 = 74-97), where *avpr1a* microsatellite length was the independent variable

and tenure was a covariate. This was done because we were unable to check the aforementioned proportional odds assumption with the predictors as continuous variables. Instead, as is typical in modeling categorical predictors, we made the categorized version of each predictor (*avpr1a* and tenure) into three dummy variables.

All statistical analyses were conducted using statistical software R version 2.13.2. The average number of offspring sired by each male and the average number of female partners with which each male sired offspring are reported as mean±1 SE. A $P \leq 0.05$ was considered statistically significant for all analyses.

Results

Avpr1a genotyping

Of the 238 adult and subadult male prairie voles that were caught on the study site, we were able to genotype 168 (70.6 %) of these males at their *avpr1a* microsatellite locus. Summed *avpr1a* lengths ranged from 1390 to 1570 bp with a median length of 1470 bp. The distribution of summed *avpr1a* lengths was statistically different from a normal distribution with proportionally more males with summed *avpr1a* lengths of 1470-1510 bp in the population than expected in a normal distribution (Shapiro-Wilk test: W=0.97, N=168, P=0.0006, Fig. 1).

Social behavior

The results of the analyses of social behavior are shown in Table 1. Male *avpr1a* microsatellite allele length did not significantly predict any of the indices of adult male space use or social

associations with females during any trapping period. Home range sizes (MSD) of adult males did not differ with male *avpr1a* microsatellite allele length. Adult male *avpr1a* microsatellite allele length did not predict either the proportion of adult females overlapped by males or the proportion of the home range overlap with the adult female a male overlapped with the most. Neither the number of adult females with which an adult male associated (AI) nor the relative strength of a male's association with his most associated female (Relative AI) was predicted by male *avrp1a* microsatellite allele length. Finally, male *avpr1a* microsatellite allele length did not predict an adult male's average residency score.

Genetic parentage assignment and reproductive success

The summary statistics for the six microsatellite loci used in the parentage analysis are shown in Table 2. We detected relatively high levels of polymorphism across loci, with 18-28 alleles per locus. A single locus (MSMM-5) exhibited a significant departure from Hardy-Weinberg equilibrium, likely due to the presence of null alleles as indicted by Cervus 3.0. However, since the frequency of null alleles at this locus was not likely to substantially bias exclusion probabilities for parentage analysis (Dakin and Avise 2004), we utilized all six loci in assigning parentage. The polymorphic information content of the loci ranged from 0.428 to 0.902.

Overall, we were able to assign parent-pairs with a 95 % confidence level for 82 of 139 (59 %) individuals first caught as juveniles, which is not uncommon in open populations of prairie voles in nature (Mabry et al. 2011). Ten of the juveniles that we were able to assign both parents using Cervus 3.0 were first trapped with an adult female (*N*=10 females) from which they were nursing. In all 10 cases the female that was assigned as the biological mother of the

juvenile using Cervus 3.0 was also the female that the juvenile was nursing from when first trapped, providing an independent corroboration of maternity for these juveniles.

Of the 144 males that were considered as candidate fathers for the parentage analysis, only 38 (26 %) of these males sired the 82 juveniles for which we could assign both a male and female parent. These 38 males that sired offspring had an average of 1.5±0.9 female mates (range 1-5). We found evidence that male *avpr1a* microsatellite allele length was associated with the number of females with which males sired offspring (N=144; t=-1.54, P=0.02). This implies that the probability of siring offspring with no females was greater among males with the shortest *avpr1a* lengths (those in the 1st quartile) than males with the longest *avpr1a* lengths (those in the 4th quartile) across all tenure categories (Table 3). Furthermore, males with the longest *avpr1a* lengths (those in the 4th quartile) were more likely to sire offspring with 1 female or more than 1 female relative to males with the shortest *avpr1a* lengths (those in the 1st quartile) in every tenure category (Fig. 2).

The 38 males that successfully sired offspring had an average of 1.7±1.2 offspring (range 1-6), with 23 males (61 %) siring only 1 offspring. Overall, we did not find evidence that *avpr1a* microsatellite length predicted whether or not a male sired offspring (N=144; z=1.57, P=0.12). However, evidence existed that *avpr1a* microsatellite length was associated with the number of offspring produced by males (N=144; t=-1.54, P=0.02). Again, this implies that the probability of having zero offspring decreased between males from the 1st quartile to those in the 4th quartile across all tenure categories (Fig. 3), meaning that males with the longest *avpr1a* lengths (those in the 4th quartile) were less likely to have zero offspring than males with the shortest *avpr1a* lengths (those in the 1st quartile). Across all tenure categories, males with the longest *avpr1a*

lengths (those in the 4th quartile) were more likely to have 1 offspring or more than 1 offspring than males with the shortest *avpr1a* lengths (those in the 1st quartile; Fig 3).

Discussion

Although previous studies have demonstrated a relationship between a male prairie vole's *avpr1a* genotype and his sociosexual behavior in laboratory (Hammock and Young 2005) and semi-natural settings (Solomon et al. 2009), this is the first study to document a relationship between a male's *avpr1a* genotype and reproductive behavior in a natural population of prairie voles. We found that a male's *avpr1a* genotype predicted whether a male sired offspring with zero, one or more than one female as well as the number of offspring sired. Contrary to our predictions, males with the longest *avpr1a* microsatellite alleles were significantly more likely to sire offspring with more than one female and to sire one or more offspring than males with the shortest *avpr1a* microsatellite allele lengths. The relationship between a male prairie vole's *avpr1a* genotype and his reproductive behavior was most evident among adult males with the most extreme differences in *avpr1a* allele length. For example, when the reproductive data was analyzed using ordinal regression after categorizing *avpr1a* and tenure into just two groups, i.e., below and above the median, instead of into quartiles, there was not an apparent effect of *avpr1a* on the number of females with which a male sired offspring or his reproductive success. Furthermore, the relationship between a male's *avpr1a* genotype and his reproductive behavior was strongest among adult males with the longest tenures (> 40 d) on the study site. This suggests that the impact of male's *avpr1a* genotype on his reproductive behavior appears relatively weak, as suggested by Donaldson and Young (2013), but can result in significant differences among males when reproductive behavior is measured over a time frame similar to

579 the average lifespan of prairie voles, illustrating the importance of long-term field studies.
580 Finally, our findings support the hypothesis that one factor responsible for the inconsistencies
581 among the previous field studies examining the relationship between male *avpr1a* genotype and
582 reproductive behavior was variability in the length of the studies.

583 Although the laboratory studies suggest that males with longer *avpr1a* microsatellite
584 alleles should be more socially monogamous in nature since they spend more time in contact
585 with their female partner and display less interest in unfamiliar females (Hammock and Young
586 2005), we failed to detect correlations between a male's *avpr1a* genotype and residency status,
587 space use or social associations with females. These findings are consistent with all of the
588 previous field studies of prairie voles that have also failed to detect any relationship between
589 male *avpr1a* genotype and indices of male space use or social association with females (Ophir et
590 al. 2008b; Solomon et al. 2009; Mabry et al. 2011). The field investigations of the relationship
591 between male *avpr1a* genotype and measures of male space use and social association with
592 females indicate that, at least for the indices that have been examined, if a male's *avpr1a*
593 genotype influences these behaviors in nature the effects are weak, relative to other genetic and
594 environmental factors that mediate these behaviors (Ophir et al. 2008b; Solomon et al. 2009;
595 Mabry et al. 2011). Perhaps indices of male social behavior with a more fine scale resolution
596 may reveal stronger correlations. Our findings regarding social behavior also suggest that the
597 greater reproductive success of males with the longest *avpr1a* alleles was not due to differences
598 in residency status, space use or social associations with females among males with different
599 *avpr1a* allele lengths.

600 Males with shorter *avpr1a* alleles spend more time investigating unfamiliar females than
601 males with longer *avpr1a* alleles in laboratory trials (Hammock and Young 2005). Therefore, we

predicted that males with shorter *avpr1a* alleles would mate with more females and engage in more extra-pair copulations (EPCs) in natural populations, resulting in these males siring offspring with more females and perhaps more total offspring. Contrary to our expectations, it was males with longer *avpr1a* alleles that produced offspring with more females and sired more offspring. In our study, we could not assess the number of matings per male but only the number of offspring sired per male that survived to trappable age. One reason that males with longer avpr1a alleles produced offspring with more females and sired more offspring may have been because the offspring of these males were more likely to survive to be trapped as juveniles and not because the males mated with more females. In laboratory studies, males with longer *avpr1a* microsatellites spent more time with their female social partner and lick and groom pups more than males with shorter *avpr1a* microsatellite lengths (Hammock and Young 2005). Since prairie voles are socially monogamous (Carter and Getz 1993) and exhibit biparental care (Solomon 1993; Lonstein and De Vries 1999), these differences in behavior may have contributed to the greater reproductive success of males with the longest *avpr1a* alleles. In the laboratory, removal of the male partner following mating significantly decreased the probability that a female would give birth compared to females whose mates were present throughout the entire pregnancy (McGuire et al. 1992; Dewsbury 1995). Laboratory-reared offspring also ate solid food earlier when their father was present (Wang and Novak 1992). Although McGuire et al. (1992) found no effect of male presence on the survival of prairie vole offspring maintained in the laboratory, which is not surprising since food is always available and there is no predation pressure, when laboratory-reared animals were released into field enclosures the presence of the female's male social partner significantly increased offspring survival (Mahady and Wolff 2002). Data from laboratory studies also suggest that males with longer *avpr1a* microsatellite alleles

may be more desirable as social partners because they would be likely to spend more time at the nest and provide more paternal care than males with shorter *avpr1a* microsatellite alleles, thereby increasing a female's reproductive success. Consistent with this hypothesis, Castelli et al. (2011) showed that females in laboratory preference trials where they were given access to males with long and short *avpr1a* alleles, spent more time with and mated more frequently with males with longer *avpr1a* microsatellite alleles. Thus the greater number of females that males with longer *avpr1a* alleles sire offspring with is not necessarily because these males engage in more EPCs. Males with longer *avpr1a* alleles may be more likely to have a social partner than males with shorter *avpr1a* alleles. Moreover, since the relationship between a male prairie vole's *avpr1a* genotype and the number of females he sired offspring with was most evident among adult males with the longest tenures (> 40 d) on the study site, long-lived males with longer *avpr1a* alleles may have more social partners over their life (e.g., due to the death of a previous partner).

Our findings on the relationship between male *avpr1a* genotype and reproduction are in contrast to those from the Solomon et al. (2009) study of semi-natural populations of prairie voles, where parentage data indicated that males with shorter *avpr1a* microsatellite alleles sired significantly more offspring with more females relative to males with longer *avpr1a* alleles. The reasons for the discrepancy between these two studies of similar length are undetermined but may result from the influence of length polymorphism at the *avpr1a* locus on male reproductive behavior being very sensitive to ecological and other genetic variation. Densities within the semi-natural enclosures were often much higher than in the Indiana population and Streatfeild et al. (2011) showed that population density was one factor that significantly affected social associations and mating patterns in prairie voles in Indiana and other natural populations. Streatfeild et al. (2011) also detected correlations between vegetation characteristics and mating

patterns. The semi-natural populations were also exposed to less predation and migration out of the population is prevented in these enclosed populations. Evidence suggests that sequence differences in microsatellites of similar length (Phelps et al. 2010), as well as, genetic polymorphism at other loci (Donaldson and Young 2013) influence the neural expression of V1aR. The variation in these genetic factors between the lab-reared descendants of wild-caught individuals from Illinois used in the Solomon et al. (2009) and the natural population of animals in Indiana in this study is unknown. Unfortunately, partially due to the complexity found in natural settings, we are unable to disentangle all the ecological and genetic variables that may have affected sociosexual behavior between these two studies.

The greater number of offspring produced by males with longer *avpr1a* alleles suggest that these males should have a higher fitness in natural populations and that, all else being equal, the longer *avpr1a* alleles in males should be favored by selection. We did detect a skew in the distribution of summed *avpr1a* lengths towards males with longer *avpr1a* alleles. The distribution of *avpr1a* alleles in this population was statistically different from a normal distribution, supporting the hypothesis that males with longer *avpr1a* alleles have a selective advantage. However, without measures of *avpr1a* allele frequencies from this population across many generations it is impossible to realistically assess how evolution is acting on allele size at the *avp1a* locus. Moreover, all things are not likely to be equal in nature. The greater reproductive success by males with longer *avpr1a* alleles may be counterbalanced by other fitness benefits associated with shorter *avpr1a* alleles in males or females. The greater reproductive success of males with longer *avpr1a* alleles also may be conditional depending on environmental factors, which may explain some of the discrepancies between this and the other

field studies examining the relationship between a male's *avpr1a* genotype and sociosexual behavior.

Nonetheless, the data from our study does suggest that a male's *avpr1a* genotype is associated with his reproductive success in nature under certain circumstances and we think this is an important finding. While laboratory studies have provided a wealth of information on the neurobiological mechanisms regulating social behavior, including behaviors associated with social monogamy (e.g., Insel 1997; Young and Wang 2004; Young et al. 2005; Bales et al. 2007), the value of these studies to understanding the sociosexual behavior of prairie voles in nature has been a matter of debate because it is unclear how these mechanisms regulate behavior within the complexities of the natural world. The importance of the current findings are that they are the first data to support the hypothesis that a male's *avpr1a* genotype is associated with intraspecific variation in the genetic mating system and male reproductive success observed in natural populations of prairie voles. The data from this study, in conjunction with the previous studies that examined the relationship between male *avpr1a* genotype and sociosexual behavior in the field, demonstrate the necessity of examining laboratory findings within ecologically relevant contexts to determine if specific neurobiological mechanisms shown to regulate social behavior in the laboratory are biologically relevant mediators of behavior in natural contexts. Furthermore, these field studies highlight the potential difficulty in extrapolating findings on the proximate basis of variation in sociosexual behavior from one natural population to another or even from one time to another within a population.

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698

699 **Ethical standards** All trapping, handling and marking procedures were approved by the Miami
700 University Institutional Animal Care and Use Committee and were in accordance with the
701 guidelines of the American Society of Mammalogists for the use of wild mammals in research
702 (Sikes et al. 2011).

703 **Conflict of interest** The authors declare that they have no conflict of interest.

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707 **References**

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Figure Captions

Fig. 1 Frequency distribution of summed length (bp) of an individual's two *avrp1a* microsatellite alleles for adult and subadult males (*N*=168) trapped in the study population

Fig. 2 The probability that males in the study population with the shortest (■ = quartile 1) and longest (■ = quartile 4) *avpr1a* microsatellite allele lengths sired offspring with zero females, one female or more than 1 female for each of the 4 tenure quartiles (1-4)

Fig. 3 The probability that males in the study population with the shortest (■ = quartile 1) and longest (■ = quartile 4) *avpr1a* microsatellite allele lengths sired zero offspring, one offspring or more than 1 offspring for each of the 4 tenure quartiles (1-4)

Table 1 Results of linear (a) and logistic (b) regressions examining the effect of male *avpr1a* microsatellite allele length on field measures of adult male space use and social associations with adult females in free-ranging prairie voles

		N	*avpr1a* genotype
Mean squared distance moved by males (a)	Period 1	40	t=0.45; P=0.45
	Period 2	42	t=-1.58; P=0.12
	Period 3	58	t=0.71; P=0.48
Proportion of females in population overlapped by each male (a)	Period 1	32	t=-0.03; P=0.98
	Period 2	39	t=-0.10; P=0.92
	Period 3	50	t=1.48; P=0.15
	Period 4	49	t=0.58; P=0.56
Proportion of home range overlap between each male and the adult female he overlapped with the most (b)	Period 1	36	z=1.20; P=0.22
	Period 2	44	z=-0.23, P=0.82
	Period 3	55	z=-1.72; P=0.08
	Period 4	49	z=0.68; P=0.50
Number of females with which a male associated (b)	Period 1	13	z=-1.22; P=0.22
	Period 2	25	z=0.23; P=0.82
	Period 3	26	z=1.80; P=0.07
	Period 4	34	z=-0.68; P=0.50
Relative strength of a male's association with the most associated female (b)	Period 1	13	z=1.21; P=0.22
	Period 2	25	z=-0.23; P=0.82
	Period 3	26	z=-1.72; P=0.84
	Period 4	34	z=0.68; P=0.50
Male average residency score during all 4 trapping periods (b)		64	z=-1.25; P=0.21

Table 2 Number of alleles (N_A), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphic information content (*PIC*) among adult male and female prairie voles for the six microsatellite loci used in the genetic parentage analysis

Locus	N_A	H_o	H_e	*PIC*
AV13	27	0.887	0.896	0.887
MOE2	18	0.818	0.859	0.846
MSMM-2	24	0.796	0.905	0.896
MSMM-3	18	0.831	0.846	0.830
MSMM-5	18	0.743	0.910*	0.902
MSMM-6	28	0.265	0.428	0.428

* Locus deviates significantly from Hardy-Weinberg equilibrium (with Bonferroni correction)

973 Fig. 1

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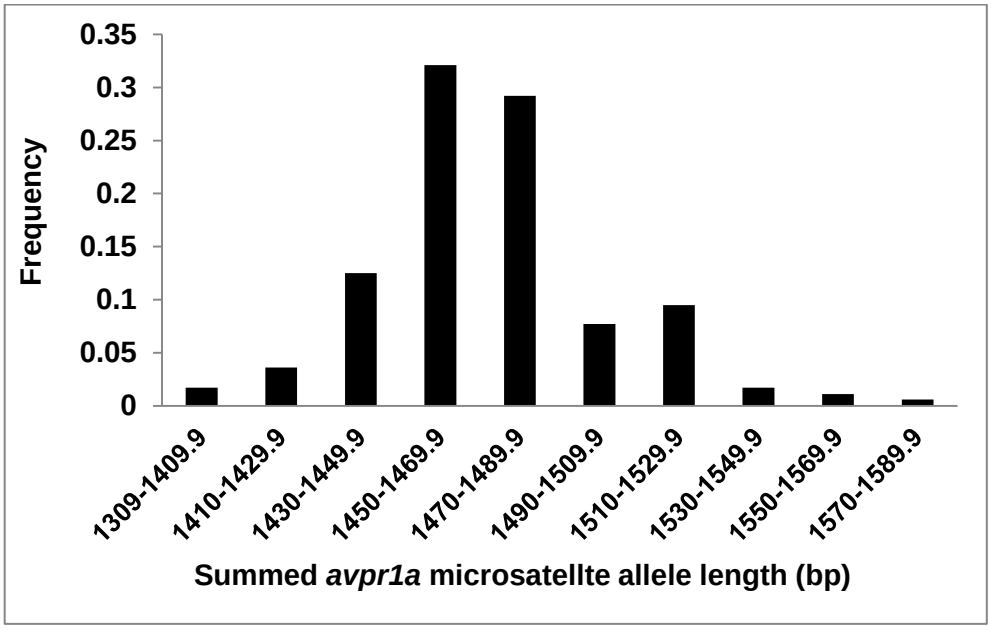

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978 Fig. 2

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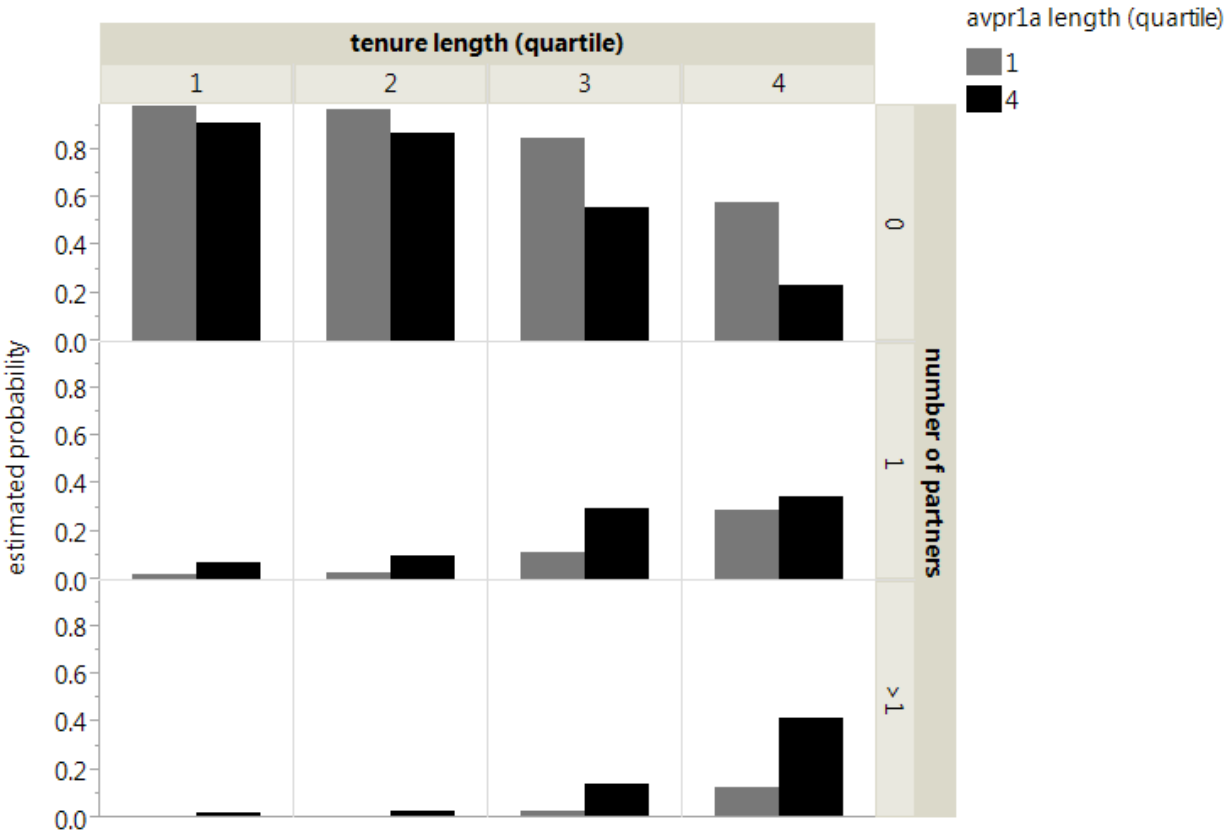

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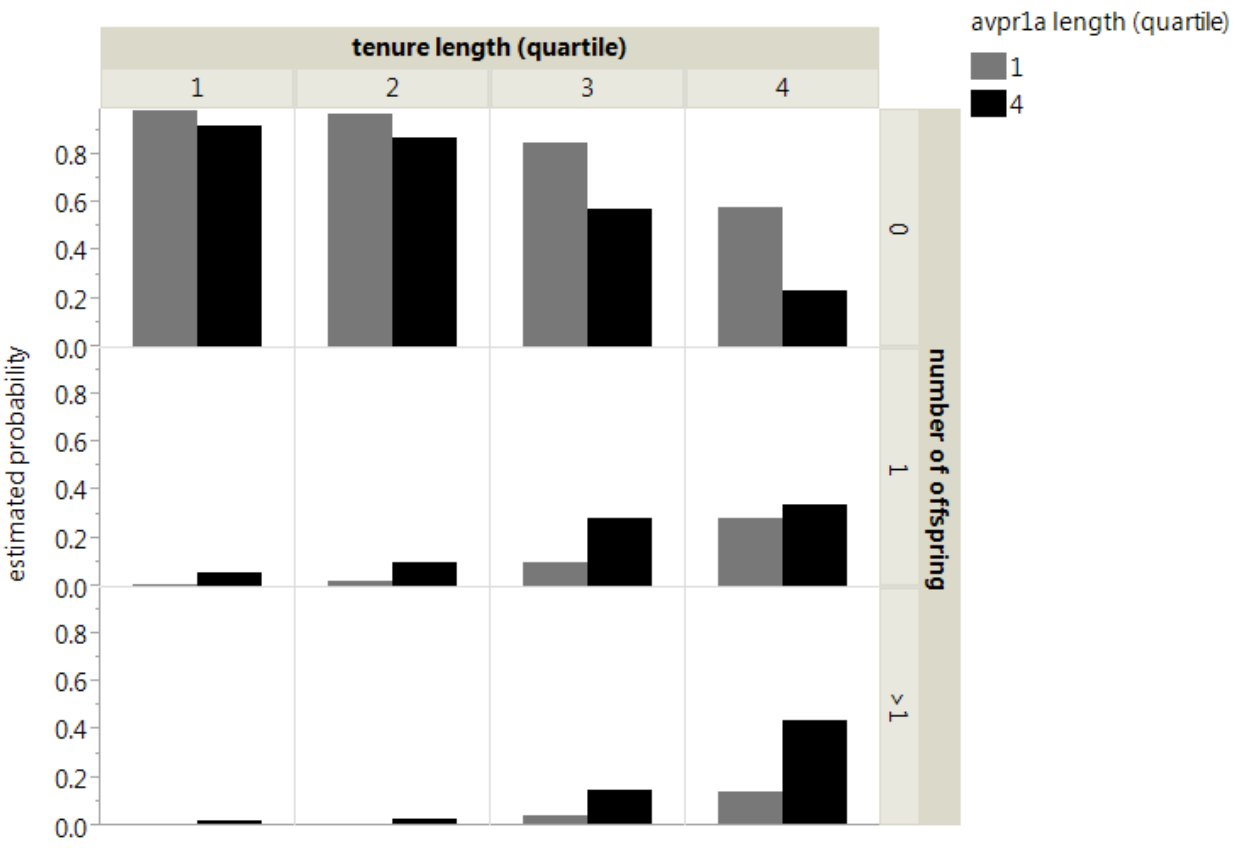
985 Fig. 3



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