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***avpr1a* length polymorphism is not associated with either social or genetic monogamy in free-living prairie voles**

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Abstract

Recent discoveries of single-gene influences on social behaviour have generated a great deal of interest in the proximate mechanisms underlying the expression of complex behaviours. Length polymorphism in a microsatellite in the regulatory region of the gene encoding the vasopressin 1a receptor (*avpr1a*) has been associated with both inter- and intra-specific variation in socially monogamous behaviour in voles (genus *Microtus*) under laboratory conditions. Here, we evaluate the relationship between *avpr1a* length polymorphism and social associations, genetic monogamy, and reproductive success in free-living prairie vole (*M. ochrogaster*) populations. We found no evidence of a relationship between *avpr1a* microsatellite length and any of our correlates of either social or genetic monogamy in the field. Our results, especially when taken in conjunction with those of recent experimental studies in semi-natural enclosures, suggest that *avpr1a* polymorphism is unlikely to have been a major influence in the evolution or maintenance of social monogamy in prairie voles under natural conditions.

Keywords

avpr1a; mating system; *Microtus ochrogaster*; monogamy; vasopressin

Recent interest in the proximate mechanisms underlying complex behaviour has led to the discovery of single genes with large effects on both neurobiological and behavioural variation (Robinson et al. 2008). Both genetic and neuroendocrine influences on a particular type of behaviour may be remarkably conserved across taxa; for example, the peptides oxytocin and vasopressin (and their non-mammalian homologues) affect similar social behaviours in vertebrate and invertebrate species (Donaldson & Young 2008; Goodson & Bass 2001). The gene that codes for the vasopressin 1a receptor (V1aR) in the mammalian brain, *avpr1a*, has been suggested to be a regulator of complex behaviour, and it is of particular interest due to its apparent effects on socially monogamous behaviour in mammals. Although most research has taken place with voles in the genus *Microtus*, particularly the socially monogamous prairie vole (*M. ochrogaster*, Hammock et al. 2005;

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Hammock & Young 2005), influences of *avpr1a* length on social behavior are not limited to rodents. A recent study found correlations between *avpr1a* polymorphism and social behavior in humans (Walum et al. 2008).

There is extensive evidence that variation in neural V1aR expression influences social behaviour both within and among species (Hammock & Young 2005, Ophir et al. 2008, Pitkow et al. 2001, Young et al. 2001). In particular, variation in V1aR expression in areas of the brain making up the 'pair-bonding circuit' has been associated with variation in socially monogamous behavior (Pitkow et al. 2001; Young et al. 2001). For example, experimental modification of neural expression of V1aR generates meadow voles (*M. pennsylvanicus*) and house mice (*Mus musculus*) that exhibit social behaviour typical of socially monogamous prairie voles, rather than the non-monogamous behaviour that is typical of their species (Lim et al. 2004, Young 1999). These and other studies demonstrate that variation in neural V1aR expression results in variation in social behaviour.

It has been suggested that both within- and among-species variation in neural V1aR is associated with a length polymorphism in the regulatory region of the *avpr1a* gene, which codes for V1aR (Hammock & Young 2005, Hammock et al. 2005, Pitkow et al. 2001). In particular, longer *avpr1a* microsatellites appear to be associated with increased V1aR expression in the pair-bonding circuit and 'more monogamous' behaviour (i.e., increased social contact) by male prairie voles in the laboratory (Hammock & Young 2005). Thus, it seems that the *avpr1a* length polymorphism influences neural V1aR expression, which in turn influences social behaviour.

However, the evidence for a direct relationship between *avpr1a* microsatellite length *per se* and social behaviour remains less clear than the evidence for a relationship between V1aR expression and social behaviour. Although laboratory studies have shown a positive correlation between *avpr1a* microsatellite length and the strength of partner preferences (Hammock & Young 2005), a recent phylogenetic study indicated that the presence of the expanded microsatellite alone does not predict the occurrence of social monogamy; most *Microtus* species possess an expanded *avpr1a* microsatellite region, but not all *Microtus* species that possess the expanded region are socially monogamous (Fink et al. 2006). A similar lack of an effect of *avpr1a* length polymorphism has been observed in a phylogenetic study in another rodent genus, *Peromyscus* (Turner et al. 2010). In light of these phylogenetic studies, the role of *avpr1a* microsatellite variation in regulating variation in socially monogamous behaviour appears to be more complex than initially hypothesized.

The results of previous studies of the effect of *avpr1a* length on social behaviour in the laboratory are intriguing, and these results lead to the question of how *avpr1a* might affect behaviour under natural conditions. The question of how *avpr1a* affects behaviour in nature is especially crucial for understanding how this gene may have been involved in the evolution of social monogamy, as has been suggested by Young & Hammock (2007). To have evolved initially, socially monogamous behaviour should convey some fitness benefit. If *avpr1a* variation is correlated with behavioural variation, we might also expect *avpr1a* variation to be related to reproductive success, with individuals possessing some variant of the gene producing more offspring. To date, two studies have examined the relationship between *avpr1a* length, neural V1aR expression, and social and reproductive behaviour in the field, using experiments conducted in semi-natural enclosures (Ophir et al. 2008; Solomon et al. 2009). In neither case did *avpr1a* length have a detectable effect on behavior indicative of social monogamy in males. However, Solomon et al. (2009) found that males with shorter *avpr1a* microsatellites mated with more females and sired more offspring than males with longer microsatellites, suggesting an effect of *avpr1a* on mating behaviour and genetic monogamy. Ophir et al. (2008) found correlations between V1aR expression and

avpr1a length, but not between *avpr1a* length and either social monogamy or reproductive behaviour. Based on the results of these two semi-natural enclosure studies, there is currently little or no support for the existence of a relationship between *avpr1a* length and behaviors involved in social monogamy in male *M. ochrogaster* outside of the laboratory.

The objective of this study was to determine whether *avpr1a* length polymorphism is related to social or reproductive behaviour of male prairie voles in unmanipulated populations. If *avpr1a* is influential in the evolution of social monogamy, we expected a relationship between microsatellite length variation, social behaviour, and mating patterns in the field under conditions similar to those under which prairie voles evolved.

Methods

Study Sites and Animals

Our study sites were located at the University of Kansas Nelson Environmental Study Area (northeast of Lawrence, KS, USA; 39°03'07"N, 95°11'27"W), and the Indiana University Bayles Road Preserve (north of Bloomington, IN, USA; 39°13'00"N, 86°32'27"W). Both sites were old fields dominated by grasses and forbs with scattered tree seedlings, and were maintained by yearly mowing to prevent the encroachment of woody plants. Field work was conducted in KS during May-June 2005, 2006, and 2008, and in IN during July-August 2006-2008, for a total of three field seasons at each site. We began field work earlier in KS because the peak prairie vole breeding season begins about one month earlier in KS than in IN (Myers & Krebs 1971; Rose & Gaines 1978), and because the KS population experiences a breeding lull in midsummer (Rose & Gaines 1978). The size of the study area varied among sites and years, from 1 ha (KS 2005) to 2.2 ha (IN 2007). However, because we used individual animals, rather than study sites, as the unit of replication in statistical analyses, differences in the area of the study sites should not affect our results.

In the field, prairie voles are typically considered to be socially but not necessarily genetically monogamous (Getz & Hofmann 1986; Ophir et al. 2008; Solomon et al. 2004; Solomon et al. 2009). Due to a recent population decline in Urbana-Champaign, IL, where much previous work on the social behavior of free-living prairie voles has taken place, we were unable to include IL voles. Social structure in the KS and IN populations is not identical to that in IL: 50% of social units in IL are male-female pairs (Getz et al. 1993) while 29% and 37% of social units are pairs in KS and IN, respectively (Streatfeild et al. unpublished data). However, all types of social units (pairs, multi-adult groups, and singletons) are present in the IL, KS, and IN populations.

Social behaviour

We characterised the social behaviour of prairie voles using live-trapping at known prairie vole nests to determine social associations. We began each field season with either one (2005-2007) or two (2008) weeks of trapping a 10 × 10 m grid laid out across the entire study site. Grid trapping allowed us to capture and individually mark animals, and obtain females to track to their nests. During grid trapping, a single Ugglan multiple capture trap (Grahnb, Hillerstorp, Sweden) was placed in a vole runway ≤ 1 m from each grid marker. All traps were set in the late afternoon and checked that evening and the following morning for five (2005-2007) or four (2008) days each week. At all other times traps were left in place but unset. We baited traps with cracked corn, covered traps with either an aluminum shield or a wooden cover board to protect trapped animals from heat and rain, and provided cotton batting as bedding material when overnight temperatures were ≤ 10° C.

We identified nest sites by either radio-tracking or fluorescent-powder tracking of live-trapped females back to their nests (see Lucia et al. 2008 for a complete description of nest

location methods). After locating nests and recording the coordinates with a hand-held global positioning system unit (eTrex Legend; Garmin, Olathe, KS, USA), we placed four live-traps within 30 cm of the entrances of each nest. Following the initial grid trapping weeks, we conducted intensive live-trapping at nests for either three (2005-2007) or two (2008) consecutive weeks to identify which adults were captured together at nests, and to capture newly emerged offspring. During nest trapping weeks, we checked traps in the mornings and evenings from Sunday evening until Tuesday evening, and again from Wednesday evening until Friday evening, for a total of 10 nest trap checks/week. During 2008, we conducted an additional four weeks (two grid and two nest) of live-trapping at both sites after the initial assessment of social behaviour. Because of trap disturbance by mammalian carnivores (raccoons, *Procyon lotor*, and domestic dogs, *Canis lupus*) during the second nest trapping period in KS, we include only trapping data from the first nest trapping period at each site during 2008. However, we did include animals trapped during the entire eight-week study period at each site in genetic analyses.

At every capture event, the individual identification number, capture location, sex, age class, reproductive condition (males: scrotal or non-scrotal testes; females: pregnant and/or lactating), and mass (g) of the captured animal were recorded. Individuals were permanently marked using unique toe-clip combinations, and tissue was preserved for later genetic analysis (see Ethical note, below). Animals ≥ 30 g in mass were classified as adults, while animals 21-29 g were considered subadults, and animals < 21 g were classified as juveniles (Gaines et al. 1979; Getz et al. 1993).

We used data from live-trapping at nests to determine the number of unique adult females with which an adult male was associated, and to quantify the strength of the associations between adult males and females. Individuals that were captured at the same nest during the same trap check were considered 'associated' during that trap check; there were 20-30 nest trap checks, and thus, 20-30 opportunities for individuals to be trapped together each year. We calculated the number of unique females with which a male was associated during nest trapping and the pairwise half-weight Association Index (hereafter AI; Cairns & Schwager 1987) between each male and every female with which he was associated. To quantify the relative strength of each male's association with the female with which he was most often associated, we calculated a Relative Association Index (Relative AI). Relative AI was calculated by dividing a male's AI with his 'most associated' female by the sum of all AIs with all females with which he associated. Relative AI is conceptually very similar to the Maximum Relative Encounter Rate (RER_{MAX}) used by Ophir et al. (2008) to quantify the relative overlap of male and female prairie vole home ranges. Values of both Relative AI and $RER_{MAX} \geq 0.5$ indicate that a male associated with a particular female more than twice as often as with all other females combined; a Relative AI of 1 indicates that the male associated with only one female. To reduce the chances of including animals that were not local residents in analyses of social behaviour, we included only adults captured > 3 times during nest trapping.

Genetic parentage analysis

To determine which adult prairie voles produced offspring together, we genotyped all live-trapped voles at six polymorphic microsatellite loci (Keane et al. 2007). We used either standard phenol/chloroform extraction techniques (Sambrook et al. 1989) or DNeasy extraction kits (Qiagen, Valencia, CA, USA) to extract genomic DNA from tissue samples and conducted polymerase chain reactions (PCR) to amplify microsatellites (see Keane et al. 2007 and Solomon et al. 2009 for further details on PCR conditions). PCR products were diluted, combined with an internal size standard (LIZ 500, Applied Biosystems, Foster City, CA, USA) and detected using an ABI 3130xl or 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Base-pair (bp) lengths of the fluorescently labeled DNA fragments

were determined with GeneMapper 3.7 software (Applied Biosystems, Foster City, CA, USA), and alleles were binned into discrete size classes using FlexiBin (Amos et al. 2006).

We conducted genetic parentage assignment in Cervus 3.0, which calculates a likelihood ratio score for each candidate parent to identify the male and female that are most likely to be the true parents of a particular offspring (Kalinowski et al. 2007; see Keane et al. 2007 for details on genetic parentage assignment). Before conducting parentage analysis, we calculated observed and expected heterozygosities at each microsatellite locus for all adult voles at each site during each year, and assessed deviations from Hardy-Weinberg equilibrium using Cervus 3.0 (Table 1). We then conducted separate parentage analyses for juveniles captured at each site during each year. Following Winters & Waser (2003), we conducted a multi-stage genetic parentage analysis, in which we first used the parent-pair option and considered all adults trapped ≤ 20 m from a juvenile's site of origin (natal nest or first grid trap site) to be candidate parents. We chose the 20 m criterion because it is approximately equal to the diameter of an average adult home range for our study populations (unpublished data). If both parents were not assigned with $\geq 95\%$ confidence in the first iteration of the parentage analysis, we then expanded the set of candidate parents to include all adults captured ≤ 40 m from the juvenile's site of origin. We restricted the set of candidate parents to those trapped within 40 m of a juvenile's natal location to minimize the possibility of spurious assignments of parentage due to chance. Finally, if we were able to assign a mother, but not a father, with $\geq 95\%$ confidence, we used the 'known mother' option in Cervus 3.0, with all adult males captured ≤ 40 m from the juvenile's site of origin as candidate fathers. We used live-trapping data, particularly captures of adult females with nursing young, to confirm the genetic assignments of juveniles to a 'known' mother. Adult females that Cervus identified as mothers at 95% confidence were always captured at least once in the same trap as the juvenile to which they were assigned, bolstering our confidence in the genetic parentage assignments.

Genetic parentage data were used to determine the total number of offspring each male sired, and the number of different females with which a male sired offspring. We considered males that sired two or more offspring with a single female genetically monogamous, and males that sired two or more offspring by two or more females were considered not genetically monogamous.

***avpr1a* genotyping**

The *avpr1a* genotypes of adult males were determined by PCR amplification of the microsatellite region of the *V1aR* gene (using primers reported in Hammock & Young 2005). Our *avpr1a* genotyping procedure was identical to that of Solomon et al. (2009), who confirmed that these primers amplified the functional copy of the *avpr1a* gene, rather than the *avpr1a* pseudogene. Because *avpr1a* contains a complex microsatellite with multiple types of repeat units (Hammock & Young 2005; Ophir et al. 2008; Young 1999), the locus does not easily lend itself to binning into distinct allele size classes. Therefore, we quantified allele length using the raw length measurements (bp) exported from GeneMapper and treated *avpr1a* length as a continuous variable. Although there is some error in estimating microsatellite length this way, we were most interested in relative *avpr1a* length among individuals, rather than absolute microsatellite length.

Statistical analyses

Because *avpr1a* has been suggested to influence inter-population variation in social behaviour (Cushing et al. 2001), we first determined whether the distribution of the summed *avpr1a* lengths of adult males differed between KS and IN with a non-parametric Kolmogorov-Smirnov test, pooling data among years within sites. We also determined

whether the distribution of microsatellite lengths at each site was statistically distinguishable from a normal distribution.

We analyzed all data on patterns of social associations and genetic parentage using parametric regressions; sample sizes were large enough to justify the use of parametric tests ($N = 40\text{--}138$ individuals), and they were at least as large as sample sizes in other field tests of effects of *avpr1a* on behaviour (Ophir et al. 2008; Solomon et al. 2009). In all analyses, the independent variable was *avpr1a* microsatellite length. We conducted separate tests of the effects of the summed *avpr1a* length, as well as the long and the short alleles individually, on behaviour. In the initial analysis, we included both *avpr1a* length and site (KS or IN) as predictors. Because we found no statistically significant effects of site on behaviour, we dropped the “site” term. However, for the sake of thoroughness, we also conducted regression analyses for each site independently (results were not qualitatively different from those of the pooled analysis). Because results were similar whether we used summed or individual allele lengths, we report only the effect of summed *avpr1a* microsatellite length on behaviour. Because we were unable to collect data on *avpr1a* length, social behaviour, and genetic mating patterns from every adult male, sample sizes vary among analyses. For example, some males were captured too infrequently for the social behaviour analysis, but sired offspring. In the case of binary responses (i.e., whether a male was genetically monogamous), we used logistic regression. Analysis of reproductive success includes all males that sired offspring, while analysis of genetic monogamy includes only the subset of successful males that sired at least two offspring. All statistical tests were conducted in PASW 18 (SPSS, Chicago, IL, USA), and were two-tailed with $\alpha=0.05$; means are presented ± 1 SE.

Ethical note

All vole trapping, handling, and marking procedures were approved by the animal care and use committees of Miami University (protocol 689), the University of Kansas (protocol 185-01), and Indiana University (protocol 10-020), and were conducted under a state of Kansas scientific permit (SC-135-2007). Collecting permits were not required by the state of Indiana.

There are multiple methods that may be used for individually marking small mammals (Gannon et al. 2007). Our study required unique, permanent marks for each individual. Non-invasive marks such as hair dyes and hair clips were inappropriate because of their temporary nature (marks are lost as hair grows or is molted). More permanent marking procedures include freeze brands, ear notches, ear tags, tattoos, passive integrated transponders (PIT tags), and toe-clips. Each of these procedures likely causes some amount of stress to the animal. Freeze brands, ear notches, and tattoos were inappropriate for this study because of the limited number of available unique marks in relation to the large number of individual animals that we trapped at each site. Ear tags are widely used to mark small mammals, but are less useful for prairie voles, which have very small external ear pinnae. The rate of ear tag loss in prairie voles may be as high as 10-16% (Wood & Slade 1990; Harper & Batzli 1996). Ear tags are lost when they become snagged on vegetation and are ripped from the animal's ear. In addition to causing pain to the animal when a tag is lost, lost tags also compromise the integrity of the data collected because individuals trapped over long time periods cannot be positively identified after tag loss. Subcutaneously implanted PIT tags allow researchers to identify individually a large number of animals. However, the implantation procedure is invasive, and implantation wounds may become infected (Harper & Batzli 1996). PIT tags were also inappropriate for this study because many animals were initially trapped as very small juveniles, and PIT tags are quite large (about the size of a grain of rice) in relation to newborn prairie voles. Toe-clipping is recommended by the American Society of Mammalogists (ASM) Animal Care and Use Committee in cases

where other marking methods are inappropriate (Gannon et al. 2007). No other marking method provided the combination of permanence, a large number of unique marks, and the ability to mark extremely small animals necessary for our study. Additionally, toe-clipping is suggested by the ASM as being especially appropriate for studies such as ours that require a small amount of tissue for genetic analysis. Finally, in a previous study, prairie voles that were toe clipped-maintained weight and survived as well as those that were ear-tagged (Wood & Slade 1990).

We individually marked animals in the field using uniquely-coded combinations of toe clips. We used a clean, sharp pair of scissors to remove toes at the second joint. Most often, 0-1 toes were clipped per foot. In rare cases, such as when an animal had already lost a toe naturally, we removed a second toe from a single foot. Approximately 3% of animals had two toes removed from a single foot. The ASM guidelines generally recommend removing only one toe per foot, but removal of two toes is permitted in unusual circumstances (Gannon et al. 2007). The ASM guidelines 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 (Gannon et al. 2007).

Results

avpr1a genotyping

We successfully genotyped 470/589 (79.8%) male prairie voles at the *avpr1a* microsatellite locus. Summed *avpr1a* lengths ranged from 1398-1574 bp in KS and 1392-1579 bp in IN. The distributions of *avpr1a* lengths from adult males differed between the KS and IN populations (Kolmogorov-Smirnov test: $Z = 1.736$, $N = 470$, $P = 0.005$). There were proportionally more males from KS with summed *avpr1a* lengths of 1430-1450 bp, and proportionally more males from IN with *avpr1a* lengths of 1450-1470 bp. (Fig. 1). In neither population was the distribution of *avpr1a* lengths statistically different from a normal distribution (Kolmogorov Smirnov test (KS): $Z = 0.81$, $N = 146$, $P = 0.52$; Kolmogorov Smirnov test (IN): $Z = 0.60$, $N = 324$, $P = 0.91$).

Social behaviour

The number of females with which a male associated was not predicted by *avpr1a* genotype (Fig. 2a; Regression: $T = 0.13$, $N = 138$, $P = 0.89$). Most males associated with 1-3 different females. The relative strength of a male's association with a particular female (Relative AI) was not predicted by *avpr1a* length (Fig. 2b; Regression: $T = 1.22$, $N = 138$, $P = 0.22$). Relative AI assesses the strength of a male's association with the female with which he was most frequently captured, and ranges from 0-1. Relative AI of 0 indicates that a male associated with no females, and Relative AI of 1 indicates that he associated with just 1 female. Relative AI values ≥ 0.5 and < 1 indicate that a male associated with more than 1 female, but was captured with a particular female more than twice as often as he was captured with all other females combined.

Genetic parentage assignment and genetic monogamy

The 6 microsatellite loci used for parentage analysis had 4-23 unique alleles in a population, with polymorphic information content ranging from 0.093-0.916 (Tables 2, 3). Overall, we were able to assign parent-pairs at the 95% confidence level for 346/601 (57.6%) of individuals first caught as juveniles.

avpr1a length was not associated with any measure of genetic monogamy or reproductive success of males. Neither the number of genetic mates, nor the number of offspring, was

predicted by *avpr1a* length (Fig. 3a,b; Regression (mates): $T = -0.59$, $N = 65$, $P = 0.56$; Regression (offspring): $T = -0.61$, $N = 65$, $P = 0.55$). Finally, *avpr1a* length did not predict whether a male that sired at least two offspring was genetically monogamous (Logistic regression: $\chi^2 = 0.39$, $N = 40$, $P = 0.53$). Overall, 48.9% (23/47) males with at least two offspring were genetically monogamous.

Discussion

Although male prairie voles displayed substantial variation in behaviour indicative of social monogamy, genetic monogamy and reproductive success, we found no relationship between the *avpr1a* genotype of males and any of these variables in free-living prairie voles in two geographically-distinct natural populations. Our results from unmanipulated populations, especially when taken in conjunction with the results of field experiments by Ophir et al. (2008) and Solomon et al. (2009), suggest that *avpr1a* length polymorphism is not strongly associated with behaviour indicative of social monogamy in prairie voles under ecologically-relevant conditions. To date, the only evidence consistent with an effect of *avpr1a* genotype *per se* on any aspect of behaviour of male prairie voles in the field is Solomon et al.'s (2009) finding that males with shorter *avpr1a* microsatellites mated with more females and sired more offspring than males with longer microsatellites in semi-natural enclosures. It is possible that the effects of *avpr1a* on mating behaviour only become evident when males sire multiple litters (~3 or more) over an extended breeding season, as in Solomon et al.'s (2009) 14-week study. It is also possible that Solomon et al.'s (2009) results from semi-natural populations were influenced by the absence of potential predators, which likely led to higher adult survival rates and extended lifespans over which males might sire more litters with more females. However, it should be noted that individual males sired multiple litters in the current study as well, with at least 24 males siring 2+ litters by different females (this estimate is conservative, because those males that sired multiple litters with the same female were not included).

What factors might be responsible for the general lack of agreement between laboratory and field studies of the effect of *avpr1a* polymorphism on social behaviour in male prairie voles? One possibility is that field and laboratory researchers are measuring different aspects of behaviour. For example, in the laboratory partner preferences are assessed by comparing the amount of time a male spends in contact with two different females, with which he has equal access (Cushing et al. 2001, Hammock & Young 2005). In the field, however, it is unlikely that a male has equal access to two females simultaneously (Getz et al. 2004) and interacts with only those females without interference from other males. Field researchers typically quantify indirect measures that are expected to correlate with partner preferences, such as home range overlap (Ophir et al. 2008; Solomon et al. 2009), or the frequency with which two individuals are trapped simultaneously at the same nest, as we have done here. Finally, in the laboratory studies, investigators examined behaviour during pair-bond formation, while in the field we may be more likely to see behaviour involved in the maintenance of pair bonds (see Bales et al. 2007). Thus, field data are fundamentally different from data collected under controlled experimental conditions in the laboratory, and results from field and lab studies may not be directly comparable (Wolff 2003).

The behavioural challenges facing free-living animals are also fundamentally different from those posed in the laboratory. In the laboratory, animals are presented with behavioural situations and choices that are likely to be much simpler than those faced in nature. In laboratory preference tests, a focal male is presented with a choice between two female conspecifics, each of which is tethered and unable to interact physically with the other 'competitor.' The behaviour exhibited by the focal animal under these conditions is unlikely to represent what the same animal might do in nature where there are multiple potential

mates, potential mates are distributed over a larger area, and multiple individuals of both sexes are able to interact over a considerably longer time (weeks, rather than hours). Further complicating matters, under ecologically-relevant conditions animals must also forage, defend their nest, and avoid predators, in addition to choosing a mate and helping to rear offspring. These competing demands may mean that in the field, potential effects of genetic variation on social behaviour may be overwhelmed by other factors.

In natural habitats, both ecological and social conditions are inherently variable through space and time. For example, many *Microtus* species experience dramatic population cycles, fluctuating from < 10 to > 600 individuals/ha (Getz et al. 2001). The social behaviour and mating choices exhibited by a male might be quite different if radically different numbers of female partners are available to him, regardless of his *avpr1a* genotype. Consistent with the possibility of a demographic effect on behaviour, Solomon et al. (2009) found that population density, but not *avpr1a* genotype, influenced space use and behaviour indicative of social monogamy by male prairie voles in semi-natural enclosures. In their study, males in low-density populations had larger home ranges and physically contacted fewer females than did males in high-density populations (Solomon et al. 2009). We were unable to test explicitly for effects of population density on behaviour, because naturally-occurring densities during our study were much lower than those reported for “high density” prairie vole populations (> 600/ha in nature; Getz et al. 1993, and up to 770/ha in semi-natural enclosures; Lucia et al. 2008). However, our data did suggest a possible effect of population density on behaviour. The apparent tendency for IN males to associate with more females than KS males may be due to higher population densities and a correspondingly higher probability of encountering more females in IN than in KS (Streatfeild et al., unpublished data).

Finally, our inability to detect an effect of *avpr1a* length on measures of either social or genetic monogamy in natural populations may result from extensive individual behavioural variation present under natural conditions. That is, a correlation between *avpr1a* length and behaviour may be present, but the pattern is obscured by a great deal of naturally-occurring behavioural variation among individuals. We cannot rule out this possibility, but sample sizes were large enough (N = 40-138) that biologically significant effects of *avpr1a* should have been evident, particularly as our sample sizes are comparable to those in other studies that detected statistically significant effects of *avpr1a* (on neural V1aR expression, Ophir et al. 2008; and genetic monogamy, Solomon et al. 2009).

Other researchers have suggested that rather than variation in *avpr1a* microsatellite length *per se*, sequence variation within the microsatellite might be important in regulating social behaviour in prairie voles (Ophir et al. 2008; Young & Hammock 2007). This suggestion is consistent with earlier observations of a positive relationship between *avpr1a* length and degree of socially monogamous behaviour if longer microsatellites are more likely to include functionally significant sequences (Hammock & Young 2005). Our data did not allow us to test the hypothesis that *avpr1a* sequence variation influences behaviour, but given the lack of agreement among recent studies of the effects of microsatellite length, both between laboratory and field studies, and among different lab studies (Hammock & Young 2005 compared to Hammock et al., 2005), sequence variation deserves further investigation. The possibility of functional sequence variation within the microsatellite control region of *avpr1a* is particularly intriguing in light of recent documentation of functional variation within the coding region of *avpr1a* in *Microtus* (Fink et al. 2007).

Recent excitement about the role of *avpr1a* in inter- and intra-specific mating system variation is primarily based on evidence from laboratory studies of voles in the genus *Microtus*, with several studies suggesting a link between *avpr1a* variation and the

occurrence of social monogamy (Hammock & Young 2005; Young 1999; Young & Wang 2004), a highly unusual mating system in mammals (Kleiman 1977). However, the data we present here indicate that *avpr1a* length polymorphism is correlated with neither social nor genetic monogamy under unmanipulated field conditions, and the presence of a normal distribution of *avpr1a* lengths in both KS and IN populations suggests that there is not a great advantage to possessing either short or long alleles (Fig. 1). Our data are consistent with other studies of social and mating behaviour under semi-natural conditions; Ophir et al. (2008) found no relationship between *avpr1a* and social or mating behaviour, while Solomon et al. (2009) found a relationship between *avpr1a*, the genetic mating system and reproductive success, but not between *avpr1a* length and behaviour indicative of social monogamy. The results of these field studies, in conjunction with a recent phylogenetic analysis of *avpr1a* polymorphism across *Microtus* (Fink et al. 2006), lead us to suggest that *avpr1a* length polymorphism is unlikely to be an important factor in the evolution or maintenance of social, and possibly genetic monogamy under natural conditions.

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Fig. 1. Frequency distribution of adult male *avpr1a* microsatellite lengths in KS and IN. The x-axis is the summed length of the two *avpr1a* microsatellite alleles for each male.

Fig. 2. Relationship between the summed length of a male's two *avpr1a* alleles and: a) Number of females with which the male associated, and b) Relative Association Index (Relative AI) between the male and the female with which he was most-strongly associated. Black dots represent males from KS, and white dots represent males from IN.

Fig. 3. Relationship between the summed length of a male's two *avpr1a* alleles and: a) Number of genetic mates, and b) Number of offspring sired. Black dots represent males from KS, and white dots represent males from IN.

Table 1

Observed and expected heterozygosities for 6 microsatellite loci used in genetic parentage analysis.

locus	Kansas						Indiana					
	2005			2006			2008			2006		
	Ho	He		Ho	He		Ho	He		Ho	He	
AV13	0.737	0.911 [‡]		0.807	0.922 [‡]		0.832	0.873		0.881	0.862 [‡]	
MOE2	0.909	0.883 [‡]		0.931	0.918 [‡]		0.874	0.888		0.756	0.857	
MSMM2	0.963	0.921 [‡]		0.966	0.938 [‡]		0.897	0.915 [‡]		0.875	0.877	
MSMM3	0.875	0.892 [‡]		0.933	0.886 [‡]		0.786	0.867		0.872	0.854	
MSMM5	0.772	0.929 [‡]		0.613	0.900 [‡]		0.870	0.934 [‡]		0.814	0.903 [‡]	
MSMM6	0.456	0.458 [‡]		0.400	0.501 [‡]		0.458	0.423		0.142	0.174 [‡]	
										0.077	0.096 [‡]	
										0.809	0.859	
										0.849 [*]	0.887	
										0.091	0.094 [‡]	

* locus not in Hardy-Weinberg equilibrium (HWE)

‡ HWE test not done by Cervus in cases where the expected number of alleles is < 5

Table 2

Summary of microsatellite data from adult prairie voles in KS.

locus	N				# alleles			polymorphic information content		
	2005	2006	2008		2005	2006	2008	2005	2006	2008
AV13	57	31	143		17	16	14	0.895	0.900	0.856
MOE2	55	29	143		15	15	16	0.864	0.895	0.874
MSMM2	54	29	136		17	17	17	0.906	0.916	0.905
MSMM3	56	30	140		13	16	15	0.874	0.862	0.853
MSMM5	57	31	138		20	19	23	0.916	0.876	0.926
MSMM6	57	30	144		9	10	10	0.437	0.466	0.409
mean				15.17 ± 1.56	15.50 ± 1.23	15.83 ± 1.74		0.82 ± 0.08	0.82 ± 0.07	0.80 ± 0.08

Table 3

Summary of microsatellite data from adult prairie voles in IN.

locus	N				# alleles			polymorphic information content		
	2006	2007	2008		2006	2007	2008	2006	2007	2008
AV13	109	283	326		14	14	13	0.842	0.847	0.860
MOE2	119	282	328		16	17	15	0.841	0.840	0.829
MSMM2	120	276	328		16	14	14	0.861	0.857	0.876
MSMM3	117	259	299		11	12	10	0.834	0.841	0.831
MSMM5	102	272	327		21	20	21	0.890	0.885	0.877
MSMM6	120	285	328		8	10	4	0.170	0.095	0.093
mean				14.33 ± 1.84	14.50 ± 1.45	12.83 ± 2.30		0.74 ± 0.11	0.73 ± 0.13	0.72 ± 0.13