Update TRENDS in Genetics Vol.23 No.5

Research Focus

On switches and knobs, microsatellites and monogamy

Larry J. Young[1] and Elizabeth A.D. Hammock[2]

[1] Department of Psychiatry and Behavioral Sciences, Center for Behavioral Neuroscience, 954 Gatewood Road, Yerkes National Primate Research Center, Emory University School of Medicine, Atlanta, GA 30322, USA
[2] Vanderbilt Kennedy Center and Department of Pharmacology, 465 21st Avenue South, MRBIII, Room 8114, Vanderbilt University, Nashville, TN 37232, USA

Comparative studies in voles have suggested that a polymorphic microsatellite upstream of the *Avpr1a* locus contributes to the evolution of monogamy. A recent study challenged this hypothesis by reporting that there is no relationship between microsatellite structure and monogamy in 21 vole species. Although the study demonstrates that the microsatellite is not a universal genetic switch that determines mating strategy, the findings do not preclude a substantial role for *Avpr1a* in regulating social behaviors associated with monogamy.

Single genes and social behavior

The idea that a single gene can markedly influence complex social behaviors has recently received considerable attention [1,2]. Microtine rodents, or voles, have proven to be a useful model system for examining the evolution, neurobiology and genetic regulation of social behavior. [3]. Previous studies in four species of vole with different social organizations – prairie voles (*Microtus ochrogaster*), pine voles (*Microtus pinetorum*), meadow voles (*Microtus pennsylvanicus*) and montane voles (*Microtus montanus*) – led to the hypothesis that the expansion of a complex microsatellite (see Glossary) in the regulatory region of a single gene, *Avpr1a* (which encodes the arginine vasopressin 1a receptor, V1aR), might have contributed to the evolution of monogamy [3,4]. By contrast, a recent paper by Fink *et al.* demonstrated that the presence or absence of this microsatellite sequence is not associated with mating systems in a larger sampling of vole species [5]. This study does not, however, challenge the idea that variation in the microsatellite or other regulatory elements of *Avpr1a* has a substantial role in generating diversity in socioemotional traits in mammalian species.

Voles, vasopressin and the pair bond

Because of the diversity in their mating systems, voles are ideally suited for studying the underlying mechanisms that regulate social behaviors. Prairie and pine voles are socially monogamous (although not necessarily genetically monogamous) and thus form selective pair bonds with their mates [6]. By contrast, montane and meadow voles are socially nonmonogamous. In the laboratory, mating facilitates the formation of a partner preference in prairie voles, and this is used as a quantitative proxy for pair-bond formation. In male prairie voles, infusion of vasopressin facilitates the formation of partner preferences in the absence of mating [7]. The distribution of V1aR in the brain differs markedly between the socially monogamous and socially nonmonogamous vole species [8]. Site-specific pharmacological manipulations and viral-vector-mediated gene-transfer experiments in prairie, montane and meadow voles suggest that the species differences in *Avpr1a* expression in the brain underlie the species differences in social bonding among these three closely related species of vole [3,6,9,10].

Microsatellites and monogamy

Analysis of the *Avpr1a* loci in the four vole species mentioned so far (prairie, montane, meadow and pine voles) revealed two genetic differences that might account for the

Glossary

Genetically monogamous: animals with a mating system in which a single male and female form a breeding pair and mate exclusively within this partnership. Genetic monogamy is rare in nature.
Microsatellit: a sequence element consisting of SSR elements, in which the repeating motif is 1–5 bases [13]. A complex microsatellite contains multiple repetitive elements interspersed among nonrepetitive sequences.
Monogamy: usually refers to a mating system in which a single male and female form a social bond, or pair bond, and mate within this partnership over an extended period of time. Mating pairs in a monogamous species typically share a nest and home range, and show biparental care. Monogamy is also used to refer to a social organization in which a male and female form a pair bond, and preferentially, but not exclusively, mate and cohabit with their partner. This social organization is generally referred to as social monogamy.
Pair bond: an enduring, selective social bond between a reproductively active male and female in a socially monogamous species. Pair bonds cannot be quantified directly but are typically assessed using a partner preference test.
Partner preference: a quantitative measure of the preferential association of one individual with either a familiar individual or a new individual. Partner preferences are used in laboratory settings to ascertain whether a social bond has formed between two individuals. A typical partner preference test uses a three-chambered testing arena. The familiar individual (e.g. the mate) is tethered to restrict its movement to one chamber, and a same-sex stimulus animal is tethered to restrict its movement to the opposite chamber. The experimental animal is placed in the middle chamber and allowed to move freely through all three chambers. A partner preference is operationally defined as a situation in which the experimental animal spends twice as much time in side-by-side contact with the familiar animal as with the new individual. The partner preference test is used in the laboratory as a proxy for the pair bond.
Simple sequence repeat (SSR): a region of DNA consisting of a relatively short motif that is repeated in tandem [13]. SSRs are prone to mutation, resulting in variable numbers of repeats in the population.
Socially monogamous: animals with a social organization in which a single male and female form a pair bond and preferentially associate and cohabit with their partner. The pair bond maintains the relationship between the breeding pair in a socially monogamous species. In such species, extra-pair copulations might occur without the disruption of the pair bond.

Corresponding author: Young, L.J. (lyoun03@emory.edu).
Available online 6 March 2007.

species differences in expression pattern in the brain [4]. First, prairie vole *Avpr1a* has been duplicated, and this type of genetic rearrangement could alter expression patterns through chromosomal-positioning effects. Second, prairie and pine vole *Avpr1a* loci contain a 430-base complex microsatellite sequence, which is composed of simple sequence repeats (SSRs) interspersed with nonrepetitive elements. By contrast, this microsatellite is present in a truncated form in the *Avpr1a* loci of montane and meadow voles, which are nonmonogamous. Transcription assays demonstrate that these microsatellite sequences influence gene expression in cell culture. These findings led to the idea that the expansion of this microsatellite in monogamous species might account for species differences in brain expression pattern of *Avpr1a* and mating strategy [11].

To test this hypothesis, Fink and colleagues examined the *Avpr1a* microsatellite of 21 *Microtus* species, in addition to the bank vole (*Clethrionomys glareolus*), the European water vole (*Arvicola terrestris*) and the house mouse (*Mus musculus)* [5]. Only three of the species examined are known to be socially monogamous. Their results replicated the original findings with regard to the prairie, pine, meadow and montane vole gene structure. Interestingly, all species examined, except for montane and meadow voles, had the longer microsatellite. Fink *et al.* then used cytochrome *b* sequences to construct a phylogenetic tree of these species (Figure 1). Their results demonstrate that montane and meadow voles are sister taxa. Thus, the presence of the longer microsatellite is the ancestral state and not the result of a recent expansion. A deletion in the microsatellite probably occurred in a common ancestor of montane and meadow voles.

The authors reasonably conclude that their data 'refute the general validity of a potential link between the presence of these genetic elements and monogamy in rodents and other taxa' [5]. However, although the data generated by Fink *et al.* clearly refute a hypothesis based on presence versus absence, their findings do not preclude the possibility that genetic variation in *Avpr1a* across species contributes to behavioral diversity, thereby influencing mating strategy. Other sources of genetic variation at this locus, including more subtle variation in the microsatellite, might contribute to the species-specific *Avpr1a* expression patterns and the behavioral traits associated with social monogamy.

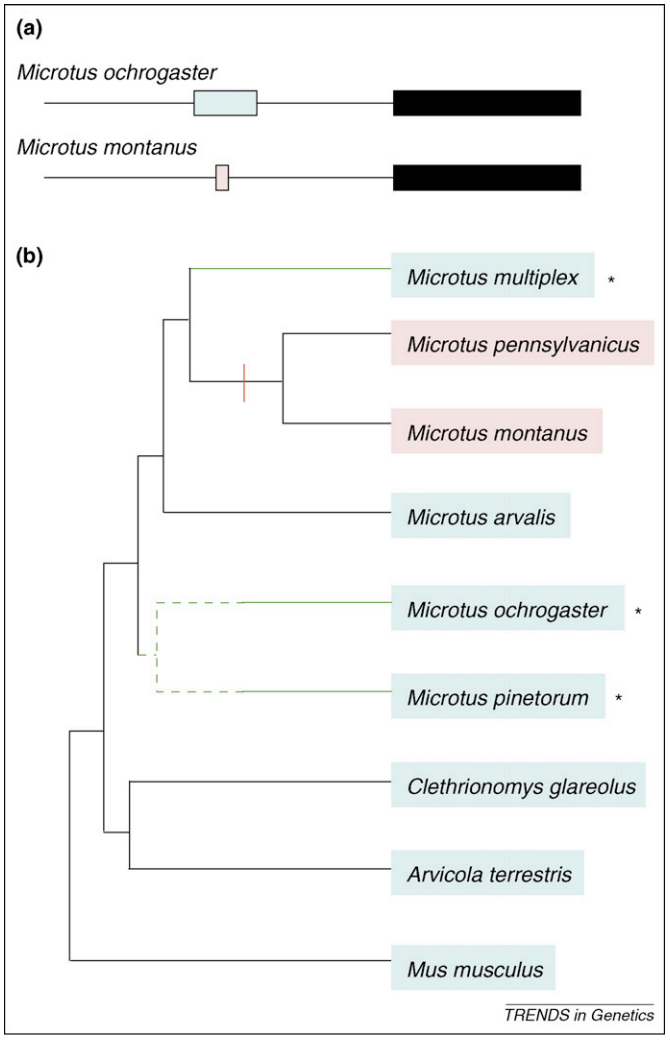

Figure 1. *Avpr1a* microsatellite structure, phylogenetic relationships and mating systems of a subset of rodent species, as reported by Fink *et al.* [5]. **(a)** Structure of *Avpr1a* and the associated microsatellite in prairie voles (*Microtus ochrogaster*) and montane voles (*Microtus montanus*). Colored boxes indicate the position and length of the microsatellite in each species. Black boxes indicate the transcribed portion of each gene. **(b)** Phylogenetic tree based on sequence homology of the gene encoding cytochrome *b* in each species. For simplicity, only a subset of the species analyzed by Fink *et al.* is presented here. Colored boxes highlighting the species names reflect the structure of the microsatellite as illustrated in (a). Asterisks denote socially monogamous species. Note that all species except montane voles (*M. montanus*) and meadow voles (*Microtus pennsylvanicus*) have the larger microsatellite. Because montane and meadow voles are sister taxa, Fink *et al.* suggest that the microsatellite was deleted in their common ancestor (red box). In addition, monogamy arose at least twice in the *Microtus* genus (green lines). It is unclear whether monogamy evolved independently in prairie voles (*M. ochrogaster*) and pine voles (*Microtus pinetorum*) or was derived from a single event in their common ancestor (broken green line), because a sister species of prairie voles, *Microtus californicus* (California vole, not shown), is socially nonmonogamous. These results illustrate that the presence (blue) or absence (pink) of the longer microsatellite does not correlate with mating system in *Microtus* species.

Switches and knobs

Fink *et al.* surmise that 'monogamy evolved independently from the presence of a putative sole genetic switch in mammals' [5]. However, these results should not be overinterpreted to imply that variation in the microsatellite does not contribute to diversity in social behavior within or across species. Kashi and colleagues have proposed that SSRs could function as evolutionary 'tuning knobs', by providing a mechanism for abundant genetic variation based on mutations that are frequent yet seldom deleterious [12–14]. Among prairie voles, there is considerable individual variation in the length and composition of the *Avpr1a* microsatellite [15]. Male prairie voles bred to be homozygous for shorter microsatellites are less likely to form partner preferences and have different *Avpr1a* expression patterns than do males with longer microsatellites [15]. Associations between microsatellite length, V1aR expression and social behavior have also been observed in heterozygous male prairie voles [16]. These observations suggest that, rather than functioning as a binary 'switch' that regulates mating strategy, more subtle polymorphisms in the *Avpr1a* microsatellite might be analogous to a tuning knob, so that frequent mutations 'tweak' brain *Avpr1a* expression patterns and, consequently, social behavioral tendencies. The data from laboratory populations of prairie voles are

compelling, but as Fink *et al.* point out, there is no evidence in any other species that variation in this microsatellite contributes to variation in gene expression or behavior [5]. A systematic analysis of sequence variation in the microsatellites of the species examined by Fink *et al.* would be useful to determine the degree of variability in microsatellite structure across *Microtus* species. It will also be important to examine the extent of microsatellite variation, and the relationship between microsatellite length and social behavioral diversity, in natural populations of prairie voles.

The vole studies that preceded the work of Fink *et al.* should not be interpreted as indicating a general linear relationship between microsatellite length and behaviors associated with monogamy. Instead, variation in microsatellite length might alter expression of *Avpr1a* in the brain in an unpredictable and brain-region-specific manner [15]. Furthermore, changes in sequence composition, rather than length *per se*, might influence expression. The relationship between microsatellite variation and social behavior depends entirely on how the variation influences *Avpr1a* expression within the neural circuits that regulate social behaviors.

Fink *et al.* also point out that the polymorphic microsatellites found upstream of primate *AVPR1A* are evolutionarily distinct from rodent *Avpr1a* microsatellites and are, therefore, unlikely to be functionally equivalent [5]. However, their equivalence might lie not in their sequence or positional homology but in their ability to alter gene expression in the brain, essentially functioning to tune expression patterns in the brain. The relationship between microsatellite length and phenotype in primates, if it exists, is likely to be much more complex than in prairie voles, as indicated by several genetic association studies in humans [17–20].

Gene networks and mating systems

The paper by Fink *et al.* also makes the important point that other genes should be considered when exploring the genetic mechanisms leading to the evolution of mating systems [5]. For example, genes in the oxytocin and dopamine systems are also involved in the regulation of partner preference. Variation in any of the genes in this network, or variation in many undiscovered genes, might result in behavioral diversity. As Fink *et al.* emphasize, *Avpr1a* is but one gene in a network of genes, the variation of which in voles contributes substantially to the behavioral phenotype of the individual and the species. Thus, independently evolved monogamy in distantly related species is likely to result from variation in other genes in the molecular pathway regulating behaviors associated with monogamy.

Future prospects

The findings reported by Fink *et al.* raise several questions for future investigation. One such question is, how generalizable is the role of *Avpr1a* in regulating behaviors associated with social monogamy? Pharmacological studies in other socially monogamous species [e.g. *Microtus multiplex*, *Peromyscus californicus* or *Callithrix jacchus* (common marmoset)] are needed to address this point. Furthermore, extensive comparative neuroanatomical studies, on the scale of the breadth of species studied by Fink *et al.*, are needed to determine the extent of the relationship between *Avpr1a* expression pattern and social behavior across diverse taxa.

The study by Fink *et al.* also raises the question of whether polymorphisms in the rodent *Avpr1a* microsatellite make any contribution to variation in expression. Knock-in experiments in which mouse *Avpr1a* is replaced with prairie vole *Avpr1a* containing either the montane vole or the prairie vole *Avpr1a* microsatellite would provide definitive evidence of whether microsatellite instability has a role in gene expression. The possibility that the *Avpr1a* duplication in prairie voles contributes to the species differences in gene expression in the brain should also be explored.

Finally, do variations in *AVPR1A* contribute to variation in human behavior and social cognition? Several studies have reported modest associations between polymorphic microsatellites in *AVPR1A* and human behavior or psychiatric disorders [17–21]. These microsatellites might be functionally linked to expression, but even if this is not the case, they could be useful polymorphic markers to test specific hypotheses regarding the functional role of *AVPR1A* in regulating human social cognition and behavior.

Concluding remarks

The detailed survey of *Avpr1a* microsatellites across vole species reveals three important points [5]. First, the microsatellite previously reported to be present in prairie and pine voles is ancestral to the shorter microsatellite present in montane and meadow voles. Second, social structure does not correlate with the presence of the longer microsatellite upstream of the vole *Avpr1a* gene. Third, it is important to consider phylogenetic relationships when comparative studies are used to identify genes regulating behavior.

It is important to emphasize that, rather than functioning as a binary switch controlling the evolution of monogamy, it is possible that variation in the microsatellite functions as a tuning knob, resulting in variation in brain expression of *Avpr1a*, which then leads to diversity in social behavior.

Acknowledgements

We thank the reviewers for insightful comments on the manuscript. We also acknowledge support from National Institute of Mental Health (NIMH) grants MH64692 and MH56897 to L.J.Y. and from National Science Foundation grant IBN-9876754.

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0168-9525/$ – see front matter
doi:10.1016/j.tig.2007.02.010

Genome analysis

V2R gene families degenerated in primates, dog and cow, but expanded in opossum

Janet M. Young and Barbara J. Trask

Division of Human Biology, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, C3-168, Seattle, WA 98109 USA

The V2R genes are expressed in the mammalian vomeronasal organ, and their products are involved in detecting pheromones. Here, we describe the evolution of the V2R gene family. We have found that the human, chimpanzee, macaque, cow and dog V2R gene families have completely degenerated. Each now contains 9–20 pseudogenes but no intact V2R genes. By contrast, opossum has 90 intact V2R genes that mostly arose by duplication after opossum and rodent lineages diverged. One V2R gene subfamily with unusual biology evolved atypically, showing limited expansion in rodents and persistence of a single, albeit sometimes dysfunctional, ortholog in all other species examined.

Introduction

The mammalian vomeronasal and olfactory systems together mediate behavioral and physiological responses to pheromones, which are chemical signals emitted by members of the same species [1,2]. Pheromone-induced activities are known to include mating, aggression towards intruders, and mothering behavior [2]. In this article, we describe the evolution of vomeronasal 2 receptor (V2R) genes, which are expressed in the vomeronasal organ together with V1R genes. The V1R and V2R gene families are related only distantly, and each encodes a set of diverse G-protein-coupled receptors that enable the detection of a broad range of ligands [1]. Other researchers have described large V2R gene families in rat (*Rattus norvegicus*) and mouse (*Mus musculus*) that mostly arose by expansion in both species after their lineages diverged [3]. However, the V2R gene family has not been examined in other species. Rodent V2R genes are found in genomic clusters, and closely related V2Rs tend to reside near one another in the genome, indicating that gene family expansion largely results from tandem duplication [3]. V2R proteins typically consist of a seven-transmembrane region, encoded by a single exon, and a long, divergent amino-terminal region, encoded by several upstream exons [4].

We developed bioinformatics tools to identify V2R genes by using sequence similarity searches, phylogenetic analysis and hidden Markov models for gene structure prediction (see the supplementary material online). We applied those tools to several mammalian genome assemblies. The mouse and rat V2R data sets that we obtain using these tools are similar to those of Yang *et al.* [3] (see the supplementary material online). They described ~200 V2R genes, of which ~60 are intact in each of the mouse genome and the rat genome. In a more recent mouse genome assembly, we found ~280 V2R genes, of which ~120 seem to be intact (Table 1), reflecting the great improvement in assembly quality in the duplicated regions that contain the V2Rs. Sequence data and genomic coordinates of the V2R genes and pseudogenes that we identified are provided as supplementary material online.

Opossum has a large V2R gene repertoire

Applying our tools to the opossum (*Monodelphis domestica*) genome, we found 86 intact V2R genes and 79 pseudogenes (Table 1). The large number of V2R genes in this species is consistent with anatomical observations that opossum has a well-developed vomeronasal system [2]. About 52% of

Corresponding author: Young, J.M. (jayoung@fhcrc.org).
Available online 26 March 2007.