White paper proposal for sequencing the genome of the prairie vole (*Microtus ochrogaster*)

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Summary: The prairie vole (*Microtus ochrogaster*) has emerged as perhaps the preeminent animal model for elucidating the genetic and neurobiological mechanisms governing complex social behavior in vertebrates (Getz and Carter, 1996; Young and Wang, 2004; Lim and Young, 2006). Prairie voles are highly affiliative, socially monogamous rodents that form enduring social bonds between mates (pair bonds) and display extensive biparental care. Other species within the *Microtus* genus are relatively asocial, uniparental, and fail to develop social bonds of any sort, providing an excellent opportunity for comparative studies focused on gene-brain-behavior relationships. The richness and diversity of the social behavioral repertoire of prairie voles and related microtine species far exceeds that of rats (*Rattus norvegicus*) and mice (*Mus domesticus*), and sets this genus apart as a unique mammalian model for discovering novel genes contributing to variation in social behavior. Biomedical research using the prairie vole model has important implications for human mental health and understanding basic human biology. In fact, remarkable parallels between the genetic regulation of social cognition and behavior in voles and man have already been described (Lerer et al., 2007; Meyer-Lindenberg et al., 2008). Discoveries made in prairie voles have led directly to novel treatment strategies for psychiatric disorders such as autism spectrum disorder (Hollander et al., 2002; Bartz and Hollander, 2006; Hollander et al., 2007; Opar, 2008). Prairie voles have also become an important animal model in evolutionary biology and behavioral ecology, with several investigators now integrating genetic, neuroscience, and behavioral ecological approaches to address important evolutionary questions (Ophir et al., 2008b; Ophir et al., 2008a). Finally, the speciation rate of *Microtus* is estimated to be ~60-100 times faster than typical vertebrates, making the genus an excellent model for understanding genome evolution. Although pharmacological and genetic manipulations are now routinely used in prairie vole research (Winslow et al., 1993; Cho et al., 1999; Aragona et al., 2003; Lim et al., 2004; Hammock and Young, 2005), the lack of comprehensive prairie vole genomic sequence seriously limits the potential of this extraordinary model system. The primary objective of this white paper is to obtain a high-quality 6X coverage draft assembly of the prairie vole genome. In addition, 2X coverage sequencing of a related asocial species, the meadow vole (*Microtus pennsylvanicus*), 0.2X coverage light sample reads from seven genetically diverse prairie voles and the establishment of a prairie vole EST sequence database are requested. These resources will complement other genomic tools that are currently being developed for prairie voles and will ensure that this species maintains it current trajectory to become one of the most powerful animal models for basic and translational behavioral research with direct implications for human mental health and human biology.

A. Specific biological/biomedical rationales for the utility of prairie vole sequence data

1. Improving human health: Biomedical research relies heavily on rat and mouse models. However, these prototypical laboratory rodent models are limited in their social behavioral repertoire. In contrast, prairie voles and related species display remarkable diversity in social behavior that can be exquisitely exploited in both the laboratory and the field to understand the social brain and its implications for mental health. Both genetic and pharmacological studies in humans have already demonstrated the translational utility and construct validity of this model for understanding human social behavior and disorders characterized by social deficits. The availability of a whole genome sequence for the prairie vole will profoundly accelerate the progress of this research. Here we review some of the active prairie vole research programs with direct relevance to human mental health.

a. Autism Spectrum Disorder: Autism spectrum disorder (ASD) is a devastating developmental disorder characterized by deficits in social cognition, social reciprocity and communication. ASD affects an estimated one in every 150 children in the U.S. and there are currently no biologically based pharmacological therapies to treat the social deficits of this disorder. However, studies in prairie voles focusing on the roles of the neuropeptides oxytocin (OT) and arginine vasopressin (AVP), in modulating social behavior have directly

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informed the treatment of ASD. In prairie voles, OT facilitates the formation of social attachments between mates (Williams et al., 1994; Cho et al., 1999). This work led to the discovery that autistic individuals have decreased plasma OT compared to control subjects (Modahl et al., 1998). Furthermore, OT infusions in high-functioning autistic subjects enhance retention of social information and leads to decreases in repetitive behaviors such as body rocking (Hollander et al., 2002; Hollander et al., 2006) (See Letter of Support from Dr. Hollander). Both lines of evidence suggest that targeting the OT system may be a viable treatment strategy for treating the social deficits in ASD as well as other psychiatric disorders with social deficits, such as schizophrenia (Bartz and Hollander, 2006). Four independent studies have now reported associations between polymorphisms in the OT receptor gene (OXTR) and ASD (Wu et al., 2005; Jacob et al., 2007; Lerer et al., 2007; Yrigollen et al., 2008).

Prairie voles have also provided the theoretical basis for the analysis of the human vasopressin 1a receptor gene (*AVPR1A*) in relation to social cognitive deficits. Prairie voles were the first species where a repeat polymorphism upstream of *avpr1a* was proposed to contribute to diversity in social behavior (Hammock and Young, 2005). There have now been three independent reports of a genetic association between similar polymorphisms in the human *AVPR1A* and ASD (Kim et al., 2001; Wassink et al., 2004; Yirmiya et al., 2006a; Yirmiya et al., 2006b). One of these studies found that *AVPR1A* polymorphisms mediated socialization skills in the subjects with ASD (Yirmiya et al., 2006a) (See Letter of Support from Dr. Ebstein). As part of an NIMH funded study to examine the role of *avpr1a* in social cognition in prairie voles, Drs. Larry Young and David Skuse (University College London) will conduct parallel studies in humans to examine the relationship between similar polymorphisms in the *AVPR1A* and social cognition in ASD patients and in the general population (See Letter of Support from Dr. Skuse). A fully sequenced prairie vole genome will greatly enhance the utility of this species as a model for ASD by providing a substrate for identifying other naturally occurring genetic variants that contribute to social cognitive deficits.

b. Addiction. The neurotransmitter dopamine, acting within the nucleus accumbens, plays a critical role in social bond formation in both male and female prairie voles (Aragona et al., 2003; Liu and Wang, 2003) In addition, dopaminergic activity in the nucleus accumbens has been widely implicated in mediating the reinforcing effects of drugs of abuse and naturally rewarding stimuli in humans (Wise, 2002). Since the reinforcing nature of drug abuse and social bonding are mediated by a common neural system, dopamine within the nucleus accumbens, prairie voles also serve as a valuable model for understanding the interaction between social relationships and drug addition. Drs. Zuoxin Wang (Florida State University) and J. Thomas Curtis (Oklahoma State University) have NIH funded research programs to investigate social- and drug-reward interaction and its underlying neural mechanisms using the prairie vole model (See Letters of Support). High quality prairie vole genome sequence will enable researchers to move from single gene approaches to approaches that simultaneously examine the entire dopamine pathway as well as to discover other neural systems that interact with this pathway.

c. Social loss, depression and heart disease. Depression is among the top five contributors to the global burden of disease, and the most costly mental health disease according to the World Health Organization. Because of their highly social nature, prairie voles have become an important model for studying the consequences of social loss or social isolation on mental and physical health. When prairie voles are chronically isolated, or separated from their pair bonded partner, they display behaviors similar to those found in depression (Bosch et al., 2006; Grippo et al., 2007b; Grippo et al., 2007a; Grippo et al., 2008). For example, pair bonded males separated from their partner for three days display high levels of passive behavior (immobility) in the forced swim test and the tail suspension tests, a behavioral response reminiscent of grieving and bereavement in humans. Several pieces of evidence suggest that the corticotrophin releasing factor (CRF) system mediates this behavioral consequence of social loss and CRF antagonists block the development of social loss-induced depressive-like behavior.

Disorders such as depression and anxiety, are recognized risk factors for cardiovascular disease. Psychological and physiological responses to stressors, and particularly reactions in the social context, play an important role in the development of affective symptoms and behaviors, and have been linked directly to cardiovascular dysfunction (Grippo et al., 2007a). Drs. Angela Grippo and Sue Carter (University of Illinois,

Chicago) use prairie voles to study the behavioral, neuroendocrine, and autonomic responses to a social stressor (social isolation), and the potential mechanisms that underlie these responses (See Letters of Support). Combined with our growing understanding of how the social environment impacts behavior, physiology, and brain function, whole prairie vole genome sequence will provide a unique opportunity to begin to understand how the genome influences or is influenced by profound social loss. Identification of genomic or epigenomic variants that contribute to depression and cardiovascular disease will help bridge a gap between genetics, psychology, and neuroscience leading to more effective treatments for and prevention of these costly, debilitating diseases.

d. Complex relationships between alcohol consumption and social behavior: In humans, alcohol consumption and social behaviors have a complex relationship. Alcohol can act as a “social lubricant", yet alcohol abuse and alcoholism may lead to severe social and marital problems, including neglect, abuse and aggression. Further, alcohol consumption (and abuse) is often observed following loss of friend or spouse. The link between social behavior and alcohol abuse has been difficult to address using animal models because most laboratory rodents don't display strong affiliative behaviors and don't show high alcohol consumption. However pilot studies indicate that prairie voles display extraordinarily high alcohol consumption in a two-bottle preference test (Ryabinin, unpublished data). Dr. Andrey Ryabinin (OHSU) has an NIH-funded research program to utilize prairie and meadow voles to address the hypothesis that neurochemical mechanisms regulating social behaviors and alcohol intake overlap and involve specific receptors for AVP and CRF. A fully sequenced prairie vole genome will allow this research to expand beyond the study of two genes. For example, transcriptome profiling of genetically variable individuals reared in different social environments will lay important grounds towards understanding the interactions between the genome and the social environment. These studies could lead to innovative approaches to the treatment of alcoholism and alcohol abuse-related disorders taking into account the social-, age- and genotype-specific particularities of individual patients.

2. Informing human biology

a. Social attachment. The formation of familial and partner bonds is a characteristic central to the human species. Prairie voles have proven to be an ideal model for understanding the genetic and neurobiological mechanisms underlying social bonding (Young and Wang, 2004). As fewer than 5% of mammals (including humans, but not *Mus* or *Rattus*) form pair bonds and exhibit socially monogamous partnerships, traditional laboratory rodent models provide little opportunity to explore the biological basis of social relationships. Like humans, prairie voles form strong social bonds and pair bonds between sexual partners that can be easily assessed in the laboratory. Non-monogamous vole species, such as the meadow vole, do not form pair bonds even after extended periods of cohabitation. Comparative pharmacological and neuroanatomical studies between monogamous and non-monogamous voles have demonstrated that OT, AVP, CRF and dopamine along with their respective receptors interact within the brain to facilitate social attachment formation in a gender-specific manner. Although there are few species differences in the distribution of the *peptides* in the brain (Wang et al., 1996), there are striking species differences in the location and density of the *receptors* for these peptides in the brain (Insel and Shapiro, 1992; Insel et al., 1994; Wang et al., 1996)(Figure 1). Prairie voles have high densities of OT and AVP receptors in the reward and reinforcement brain regions, nucleus accumbens and ventral pallidum. Pharmacological studies have demonstrated that these receptor populations regulate pair bond formation. Further, when viral vector gene transfer is used to over-express the prairie vole AVP receptor in the ventral pallidum of the meadow vole, their social behavior is transformed such that they develop partner preferences (Lim et al., 2004). Brain imaging studies in humans have revealed that related reward structures

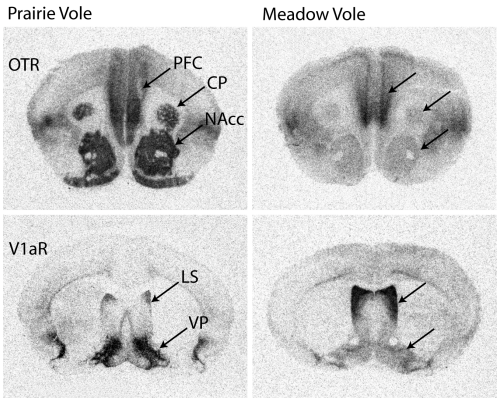

Figure 1. Species differences in oxytocin receptor (OTR, top row) and vasopressin receptor (V1aR, bottom row) of the monogamous prairie vole and non-monogamous meadow vole. These differences illustrate the power of comparative studies in these species.

are activated by viewing photographs of loved ones (Bartels and Zeki, 2000, 2004; Fisher et al., 2005). It is likely that a wide range of genes contributes to the formation, maintenance, and regulation of bonding behavior, and that there will be considerable overlap in those genes in voles and man.

One of the most valuable features of the prairie vole system is the genetic and behavioral diversity of natural populations and laboratory colonies. For example, in the field 40% of male prairie voles do not display behaviors typical of monogamy. In the laboratory, some males bond quickly while others do not. Dr. Larry Young's laboratory is selectively breeding lines of prairie voles that differ in their ability to form partner preferences. The availability of genome sequence and a high density SNP panel will facilitate planned QTL mapping studies to identify genetic loci contributing to this variation in social bonding. Furthermore, microarrays made possible by the EST sequences will facilitate the identification of genes within specific brain regions whose transcript levels are associated with the ability, or lack there of, to form social attachments.

b. Parental care. Like humans, both virgin and post-partum female adults display nurturing behavior toward infants. However, there is remarkable individual variation in this alloparental behavior. Among prairie voles, 60% of adult females display spontaneous maternal nurturing while 40% either ignore or attack pups (Lonstein and De Vries, 2001; Olazábal and Young, 2006a,b). This intra-species variation in nurturing behavior provides a unique opportunity to elucidate the genetic and neurological mechanisms underlying this behavior. For example, virgin female prairie voles that display maternal behavior toward foreign pups have higher densities of OT receptor in the nucleus accumbens than non-maternal females (Olazábal and Young, 2006b, a). Unlike *Rattus* and *Mus*, male prairie voles also contribute substantially to rearing their young and are an important model for understanding the genetic and neurobiological basis of paternal care. Dr. Geert De Vries (UMass Amherst) and colleagues have exploited this model to demonstrate that AVP stimulates paternal behavior in males. Specifically, his group has shown that cohabitation with a female results in an increase in AVP transcription and that AVP receptor antagonists can block this behavior under certain conditions. Dr. Maryam Bamshad (Lehman College) investigates how experience alters the circuitry underlying paternal care in prairie voles (See Letters of Support). It is clear that multiple pathways facilitate parental behavior in voles and full genomic sequence will greatly expedite the identification of these interacting genes and networks.

c. Early life experience affects adult social behavior. In humans, rats mice and voles, there is now growing evidence that the quality of early life nurturing can have life-long influences on adult social behavioral tendencies, presumably through epigenetic effects. Drs. Karen Bales (Univ. of California, Irvine) and Sue Carter (Univ. of Ill.) have convincing evidence that the effects of early social experience affect adult social behaviors including alloparental behavior, the capacity to form a partner preference, the tendency to show same-sex aggression, and reactivity to stressors (See Letters of Support). The behavioral changes appear to reflect modifications in several brain systems including OT expression (Yamamoto et al., 2004) and AVP receptor expression (Bales et al., 2007). Thus the prairie vole brain is exquisitely sensitive to the influence of social experience which shapes the expression of behaviorally relevant genes. The molecular mechanisms by which social experience alters brain gene expression and thereby behavior is unknown. However, it is clear that the prairie vole is an excellent model for understanding how early life experience shapes our later socio-emotional traits. The availability of genome sequence will facilitate epigenomic studies to understand this phenomenon.

d. Social Cognition. Social bonding in monogamous species is a complex cognitive process involving social motivation, the neural regulation of social cues and attachment. Partner preference formation in prairie voles provides an opportunity to discover mechanisms regulating a wide range of social cognitive processes. This is apparent in the human pharmacological studies that followed from the discovery in prairie voles that OT enhances partner preference formation. Intranasal OT infusion enhances interpersonal trust in economic based tests (Kosfeld et al., 2005), decreases amygdala activation while viewing socially threatening stimuli (Kirsch et al., 2005) and, particularly relevant to autism, enhances the ability to read social signals from subtle facial expressions (Domes et al., 2007). All of these studies were performed in humans and are consistent with earlier work in prairie voles. This is proof of principle that discoveries made possible by the availability of prairie vole genome data may indeed be directly relevant to human social cognition.

e. Sex differences in social behavior. Males and females of many species display sex-specific behaviors (sexual behavior, territorial aggression, parental care) that are mediated by sex differences in neurochemistry and gene expression. Dr. Geert De Vries has pioneered the research in sex differences in the brain and has shown that AVP is one of the most consistently sexually dimorphic molecules in the brain, with male brains having significantly more AVP than female brains (De Vries and Panzica, 2006). Prairie voles are unusual among rodents in that both males and females care for their young. Dr. De Vries postulates that while sex differences in AVP mediate sexually dimorphic behaviors in many species, in prairie voles this system actually leads to convergence in behavior in this biparental species. That is, AVP promotes paternal nurturing in males that is comparable to maternal behaviors typically seen only in female rodents. Work from Dr. Joe Lonstein's laboratory focuses on how early-life exposure to steroid hormones influences the emergence of sex differences, or similarities, in the prairie vole brain and social behavior. His work on steroid-sensitive AVP and dopamine systems, and how they contribute to sex differences or similarities in parenting and other social behaviors, reveals developmental processes that are unique among rodents. The Lonstein lab also recently discovered a species- and sex-specific population of dopaminergic cells in the prairie vole amygdala. They hypothesize that the very high number of these cells in male prairie voles, but not in female prairie voles or meadow voles of either sex, implicates them in male prairie vole pair bonding and paternal behavior (See Letter of Support). Prairie vole genome sequence will allow for rapid screening for genes within specific brain regions that are differentially expressed between the sexes and will greatly facilitate the development of siRNAs and other molecular tools to manipulate the expression and localization of genes. These resources will have immediate and profound impacts for understanding how sex differences in the brain can shape species-typical behaviors relevant to human behavior.

f. Polymorphic regulatory elements generate diversity in sociality. Variations in gene regulatory elements are thought not only to distinguish our species from apes, but also contribute to variation within humans for specific traits (Haygood et al., 2007). Since species differences in social bonding between monogamous and non-monogamous voles are associated with species differences in *avpr1a* expression patterns in the brain, Dr. Young compared the *avpr1a* gene structure in social prairie and asocial montane voles. Although the coding sequence of *avpr1a* is ~99% conserved between vole species, the prairie vole locus has a 430 bp, complex microsatellite located 760 bp upstream of the transcription start site which is absent in the montane vole (Young et al., 1999). Proximal regulatory elements in the *avpr1a* gene appear to be responsible for species-typical *avpr1a* expression pattern in the brain since transgenic mice expressing the prairie vole *avpr1a* locus including 2.2 kb of 5' non-coding sequence exhibit prairie vole-like V1aR distribution patterns and display increased affiliative behavior compared to non-transgenic controls (Young et al., 1999). Further when the prairie vole microsatellite is replaced with the shorter montane vole sequence in cell culture, transcription of *avpr1a* is altered in a cell type-specific manner (Hammock and Young, 2004). It should be noted that the simple presence or absence of this microsatellite is not associated with social organization in other *Microtus* species (Fink et al., 2006; Young and Hammock, 2007), suggesting that variation in other genes or regulatory elements also contribute to natural variation in social behavior. Within prairie voles, this microsatellite element is polymorphic and evidence suggests that this polymorphism directly contributes to individual variation in V1aR distribution in the brain as well as in social behavior (Hammock and Young, 2005). Remarkably, similar polymorphic repeats are found upstream of the human *AVPR1A*, and Dr. Richard Ebstein's group has shown associations between microsatellite length and social cognition and altruistic behavior as well as *AVPR1A* expression in the brain (See Letter of Support from Dr. Ebstein). These observations illustrate how taking advantage of the genetic diversity in laboratory populations of prairie voles can lead to exciting insights into how variation in gene regulation affects behavior in rodents as well as in humans. These studies further demonstrate the necessity for acquiring full regulatory and coding sequence in order to begin to understand how variation in genetic regulatory elements contributes to diversity in behavior. A high-quality sequence of the prairie vole genome will allow for the use of powerful technologies such as ChIP-Sequencing to begin to understand variation in gene regulation among individuals, how sequence variants within regulatory elements might affect transcriptional machinery and the number of genes that are subject to variation within *cis*-regulatory regions.

3. Expanding our understanding of basic biological processes relevant to human health

a. Adult neurogenesis. Like humans, prairie voles display newly proliferated neurons in select brain regions, although the significance of this neurogenesis is unclear. Studies in both prairie and meadow voles have shown that while adult neurogenesis is influenced by factors such as gonadal steroid hormones and stress hormones, it also has a strong environmental component and can be influenced by mating, reproductive status, and exposure to pups (Galea and McEwen, 1999; Ormerod and Galea, 2001; Smith et al., 2001; Fowler et al., 2002; Fowler et al., 2003; Fowler et al., 2005; Ruscio et al., 2008). Manipulation of the social environment (either social isolation or exposure to males) in adult female prairie voles alters neurogenesis in brain regions involved in regulating social behavior. With full genome sequence, prairie voles will be a valuable animal model for discovery of the regulatory and genetic mechanisms that underlie adult neurogenesis as it relates to social behavior.

b . Comparative genomics of rodents. Comparisons of full genome sequences of *Mus* and *Rattus* have provided numerous insights towards understanding mammalian genome evolution. The genomes of muroid rodents appear to be evolving at a much faster rate than primate genomes and possess numerous oddities such as large chromosomal rearrangements, a great number of gene expansions and other previously unknown genomic features such as breakpoint reuse. Are mice and rats genetic oddities, or do other rodents share similar patterns of genome and sequence evolution? Currently, there is still a large phylogenetic gap between these well-described muroid lineages and humans or other non-human primates. Although full genome sequencing of several other muroid species is in process (or in queue), many of these species are not amenable to laboratory studies and have little value as a biomedical model. One exception to this is the forthcoming full genome sequences of species within the genus *Peromyscus*. Like *Mus* and *Rattus*, *Peromyscus* and *Microtus* are sister taxa that diverged from one another in the past ~20 million years, are invaluable models for laboratory research pertaining to human health, and provide a unique opportunity to understand how natural genetic variation influences phenotypes. Genomic comparisons between these related species will shed further light on muroid genome evolution in a manner that can be directly compared to *Mus* and *Rattus* (See Letter of Support from Dr. Hoekstra). In addition, full genome sequence of species from both *Peromyscus* and *Microtus* will serve as valuable species intermediates linking more distantly related muroid rodents and primates (see Figure 2).

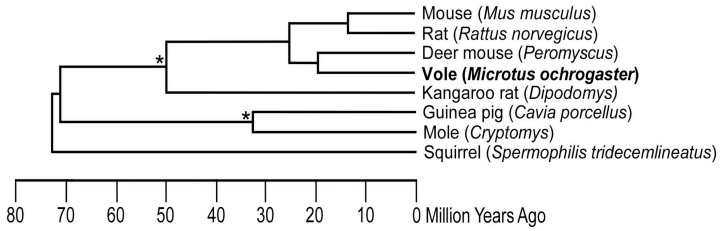

Figure 2. Phylogeny of rodent genomes selected for whole-genome sequencing. (Divergence dates and tree topology are based on (Eizirik et al., 2001; Steppan et al., 2004; Murphy et al., 2007)
* Indicates nodes that represent a minimum divergence estimate.

c. Comparisons of genome structure, organization, and evolution. In addition to providing a unique point of comparison with muroids, voles are an especially interesting animal model for the study of genome evolution. Among *Microtus* species, karyotypes are exceedingly plastic with diploid chromosome numbers ranging from 2n=17 to 2n=64 (Maruyama, 1981). At the sequence level, the rate of evolution within *Microtus* appears to be faster than any other rodent and speciation rates are estimated to be ~60-100 times higher than most vertebrates, resulting in the emergence of over 60 vole species in ~two million years. These observations are extremely intriguing given that the majority of *Microtus* species are physically nearly indistinguishable. Drs. Andrew DeWoody (Purdue University) and Deb Triant (LSU) sequenced the mitochondrial genome of a European vole, *M. rossiaemeridionalis* and discovered that rates of mitochondrial evolution were the highest within the genus *Microtus* relative to other mammalian taxa (Triant and DeWoody, 2006). Voles also have a much higher rate of mitochondrial insertions into the nuclear genome (numt transfers) than mice or rats (Triant and DeWoody, 2007, 2008) (See Letters of Support). A whole genome assembly of the prairie vole as well as low coverage sequence from the meadow vole will therefore provide a novel perspective on rates of molecular and chromosomal evolution in mammals.

d. Sex determination and sex chromosome evolution. In addition to their rapidly evolving karyotype and genome, voles are also an evolutionary outlier with respect to their sex chromosomes. First, at least twelve vole species possess "giant sex chromosomes" where large blocks of heterochromatin have been added to the sex chromosome pair. In *Microtus agrestis*, ~20% of the genome is found on the X chromosome (Nanda et al., 1988)! Other species such as *Microtus cabrerae* possess length polymorphisms of sex chromosomes as a result of deletion mutations of heterochromatin blocks (Burgos et al., 1988; Modi et al., 2003). Likewise, although prairie voles possess a typical mammalian sex determination system (XX vs. XY), other vole species possess bizarre sex chromosome structures. For example, in the creeping vole, *Microtus oregoni*, both sexes are gonosomic mosaics where males are OY/XY and females are XX/XO and in the mole vole, *Ellobius lutescens*, both males and females are XO. Thus, the X chromosome in these species is completely sheltered from recombination. Finally, many other sex chromosome oddities exist among voles including females that possess autosomal copies of the SRY gene (the male sex-determination locus; (Fernández et al., 2002), conspecific males and females that possess differing numbers of chromosomes (Charlesworth and Dempsey, 2001) and nonrandom X chromosome inactivation in hybrid crosses (Nesterova et al., 2001). A full genome sequence of the prairie vole will provide an exciting starting point for detailed sequence analysis of the bizarre sex chromosome systems of voles (See Letter of Support from Dr. Bachtrog).

e. Behavioral/ecological correlates of sociality. The original classification of prairie voles as highly social and monogamous was based upon field studies conducted in prairie voles in Illinois (Getz et al., 1981; Getz et al., 1993). However, there are significant population differences in social behavior among prairie voles from different geographical ranges. For example, prairie voles from Kansas are less social and display lower levels of physical contact between adult males and females, less spontaneous alloparental and parental behavior, and are more aggressive than Illinois prairie voles (Roberts et al., 1998; Cushing et al., 2001). However, Kansas voles are more socially and genetically monogamous than voles in an Indiana population (Solomon and Keane, 2008). Dr. Bruce Cushing has determined that some of these population differences are associated with differences in estrogen receptor expression in the brain (See Letter of Support). Drs. Steve Phelps (University of Florida) and Alex Ophir (University of Florida) have also been examining neural correlates of individual variation in mating strategy in prairie voles in naturalistic enclosures (See Letters of Support). Their findings suggest that variation in *avpr1a* expression in specific brain areas actually predicts reproductive success as a function of mating tactic and partner fidelity (Ophir et al., 2008). Drs. Phelps and Ophir are now using viral vector mediated siRNA to manipulate gene expression in the brains of prairie voles in these naturalistic enclosures. The availability of full genomic sequence will allow for siRNA constructs for any gene of interest to be developed in a rapid manner and can be coupled with transgenic techniques to create voles that lack tissue-specific expression of a given gene. These tools will have profound impact on this powerful approach to study the function of individual genes in a natural context.

Drs. Nancy Solomon and Brian Keane at Miami University have also been studying prairie vole social behavior in the lab and field. Their laboratory maintains detailed information on mating strategy (e.g. whether the male is a "wanderer" whose territory overlaps with many females, or is a "resident" whose territory overlaps with a single female) as well as paternity in wild populations of prairie voles from various geographical locations. In addition to this extensive behavioral data set, tissue samples are routinely archived. In fact, detailed behavioral data as well as DNA have already been collected from 300 Kansas and 800 Indiana prairie voles (See Letter of Support). Full genome sequence will allow for the design of high-density SNP arrays which can be used to identify chromosomal loci that are associated with mating strategy. These studies in natural populations will complement the selective breeding QTL studies being conducted in the laboratory of Dr. Young. Genome association studies will be complemented by microarray expression studies, made possible by the EST sequence database, to identify novel genes contributing to the variation in behavior.

4. Providing additional surrogate systems for human experimentation

As has already been elaborated on in previous sections, the prairie vole has been a premier model for human social cognition and mental health research. Several discoveries made in the OT and AVP systems of the prairie vole directly translate to human biology and mental health. If the research in these candidate genes have already made this impact on mental health and human biology, it is clear that future discoveries facilitated

by the development of genomic sequence and resources may have equally important implications for understanding our own species.

5. Facilitating the ability to do experiments

Prairie voles provide a truly unique opportunity to understand the interaction of the social brain and mental health, ranging from autism and addiction, to depression and alcoholism. Unfortunately, the lack of sophisticated, genomic resources for this species has left studies on social bonding limited to a handful of candidate genes. As we have already outlined above, the following genomic resources will undoubtedly facilitate rapid discovery of new genes and will catapult the prairie vole as unique mammalian model to ultimately link the genome to the brain and to social behavior and cognition.

a. 6X draft coverage of the prairie vole genome: Research in prairie voles has demonstrated that subtle genetic variants in non-coding DNA can have profound effects on social behavior. Thus, it is imperative that high quality sequence of both coding and non-coding regions be developed for this species. A high quality genome sequence will 1) immediately provide important insights into the abundance and distribution of genes, gene families and repetitive elements, 2) provide a readily available substrate for the isolation and/or amplification of specific sequences for research ranging from biomedical applications to ecology and evolutionary biology, 3) enable whole genome comparisons of voles to that of other rodents and other more distantly related mammalian species and 4) provide a quality starting material for the development of subsequent genomic tools such as tiling arrays, SNP chips, genome-wide methylation screens, etc. that comprehensively span both coding and non-coding elements. A high quality draft assembly will immediately allow for discovery-based genome research and will expedite the development of molecular tools to dissect the genome at a finer scale in ways that targeted sequencing or BAC end-sequencing can not accomplish.

b. 0.2X low-coverage sequencing of seven genetically diverse prairie vole individuals. Prairie voles are unique among other mammalian biomedical models in that they are routinely maintained as outbred populations and individuals vary remarkably in their behavioral repertoires and basic biology. To take full advantage of this unique aspect of prairie vole research, low-coverage sequencing of seven prairie vole individuals will allow for rapid SNP discovery which will aid in mapping the loci that contribute to the behavioral phenotypes that we have described above.

c. 2X draft coverage of the asocial, meadow vole (*Microtus pennsylvanicus*). As described above, comparative studies between the highly social prairie vole and the relatively asocial meadow vole have led to intriguing insights into the genetic mechanisms that lead to species diversity. A light coverage draft assembly of the meadow vole will allow for rapid, targeted comparisons of gene regions and aid in the ability to identify and amplify homologous genes and gene regions in more distantly related vole species.

d. EST libraries. To facilitate in genome annotation and to provide immediate isolation and identification of tissue-specific transcripts, EST libraries from several adult tissues including liver, ovaries, testes, as well as a whole brain from both a male and a female vole will be invaluable. In addition, as many important cell types are only found within small regions of the brain, transcripts from small, yet biologically critical brain regions have a high probability of not being represented in whole brain EST libraries. Thus, EST libraries specific to the mid/hind brain, the striatum, the amygdala and the hypothalamus will be invaluable for understanding the connections between genes and neural circuitry. Investigators will be able to develop and utilize DNA microarrays from these EST sets and are already poised to begin mining the databases for sex-specific transcripts, brain region-specific transcripts, etc.

B. Strategic issues in acquiring vole sequence data

1. Demand for new sequence data.

There are a growing number of social neuroscience, genetics, physiology, evolution and behavioral ecology laboratories using prairie voles as their animal model of choice. In the past five years, the number of published manuscripts using voles has increased considerably and currently, 15 NIH grants as well as five active NSF grants have been awarded to prairie vole researchers. Over 25 vole researchers are enthusiastic about

developing genomic resources for *M. ochrogaster* and have provided letters of support (appended). In addition, there are more than 75 trainees in the prairie vole research community. These young researchers are aware of the power of genomic approaches and are poised to begin utilizing the genome sequence and resources being requested. With the availability of full genome sequence, we fully anticipate the next generation of prairie vole researchers will be genomically enabled and will propel this animal model as the premier animal model for understanding social neurobiology, ecology and evolution. The prairie vole community is growing quickly and we have already obtained funding through internal mechanisms to hold a Prairie Vole Meeting at Emory University in the Spring of 2009. We expect representatives from approximately 20 vole labs to attend including P.I.'s and young researchers. Further, a prairie vole genome will provide unique opportunities for genome-wide comparisons of *Peromyscus* and other muroid rodents. Several of our colleagues from the Peromyscus community have voiced their support of the prairie vole genome sequence.

2. The suitability of the prairie voles for experimentation.

a. Suitability of the prairie vole for experimentation. Prairie voles are intermediate in size (typically 30-60g) compared to laboratory mice and rats. They are easily maintained in standard rodent viviariums and have a gestation period of 21 days and a generation time of approximately three months. Affiliative behavior, social bonding, parental care and other classic behaviors can be easily studied and quantified in the laboratory using the same tests and equipment as is used for mice and rats.

One of the major advantages of prairie voles is that they readily adapt to laboratory environments where traits can be studied under controlled conditions. To take advantage of natural variation in social behaviors, at least 12 laboratory colonies of prairie voles in the U.S. are traditionally maintained as outbred populations. For example, the prairie vole colony maintained by Dr. Larry Young at Yerkes National Primate Research Center is systematically outbred and within the past year, wild-caught voles have been crossed into the colony to ensure that considerable genetic variation is maintained among individuals. This genetic variation permits us to generate breeding regimes to select for traits of interest. Currently, two lines are being generated where males are selected for their propensity to form a pair bond with their mate. Because outbreeding strategies are not ideal for some types of genetic analyses, several lines of inbred animals, including a line with a spontaneous coat color mutation are also being maintained. In addition to prairie voles, many laboratories also maintain populations of meadow voles, or other vole species that have proven invaluable for comparative studies of social behavior. Not only can voles be studied in the laboratory, but they can also be studied in their natural environments or in semi-natural enclosures allowing for a more realistic measure of behavior. The vole research community has a long history of routinely sharing animals and genetic resources. As our resources accumulate, we anticipate the establishment of a central stock center for both animals and genetic/genomic resources much like the *Peromyscus* community has developed.

b. Genetic manipulation. At least four laboratories have already used viral vector approaches to manipulate gene expression in the prairie vole brain (Cushing, Phelps, Wang, Young). Adeno-associated- or lenti-virus vehicles have been used to express siRNAs site-specifically to knock-down gene expression within the prairie vole brain. In addition, within the past several months, Dr. Young's laboratory in collaboration with Dr. Anthony Chan has successfully used lentiviral vectors to create the first transgenic prairie voles that express the GFP gene driven by a ubiquitin promoter (See Letter of Support from Dr. Chan). We anticipate that siRNA transgenics with temporal and brain region-specific control will greatly enhance the ability of prairie vole researchers to determine the roles of specific genes. Furthermore, it will be possible to conduct these experiments in semi-natural environments to determine the contribution of individual genes to behavior in natural conditions.

c. Other genomic resources. Drs. Larry Young and James Thomas have acquired NIMH funding to generate a 10X coverage BAC library. The BAC library is currently being constructed by Dr. Pieter de Jong (http://bacpac.chori.org) and we expect it to be completed within the next four months. Once the BAC library is in hand, the library will be probed using pre-designed and validated 'universal' overgo probes developed by the Thomas lab specifically for screening rodent genomic libraries (Kellner et al., 2005) to rapidly isolate BAC clones that are of immediate interest to the prairie vole and neuroscience communities. Dr. Young has also received funding from Autism Speaks (www.autismspeaks.org) to generate SNP and microsatellite markers

from a random sampling of BAC clones to make a first-generation linkage map that can be anchored to other fully sequenced rodent genomes. BAC clones will be made publicly available and as sequences and markers are obtained, they will be deposited to GenBank. In addition to these resources, Dr. Miguel Pita (Autonomous University of Madrid) will use the BAC clones identified above to generate comparative cytogenetic maps of both prairie and meadow voles.

3. Rationale for complete sequencing of the prairie vole.

As described above, a high quality, draft assembly of *M. ochrogaster* genome will provide essential tools for prairie vole research making it an even more valuable model for studying human health and disease. Beyond this, full genome sequencing will enable informative whole genome comparative analyses to other mammalian and more distant vertebrate models. An active biomedical research community that is poised to integrate genomic data into a variety of experimental paradigms will immediately benefit from high quality genomic sequence of both coding and regulatory regions.

4. Cost of sequencing the genome, state of readiness of organism's DNA for sequencing

a. Size of the genome and repeat structure. The prairie vole genome is similar in size to *Mus* (~3000 Mb) and is predicted to contain a similar fraction of repetitive elements as other rodents.

b. Quality of sequence product needed. It is highly desirable that a stand-alone assembly of a quality level equivalent to a ~6x draft (Sanger sequencing) be generated using a method that is deemed most cost-effective to achieve this goal. As described in earlier sections, it is important that a 'full-draft' versus just a low-coverage 'comparative' draft, be produced. In addition, since other genomic resources, i.e. a genetic linkage map and a BAC library for cytogenetic mapping, for anchoring the sequence contigs to specific chromosomes will be available for voles, it will be possible to anchor and order most of the sequence generated in a full-draft assembly back to specific chromosomes.

c. Sequencing strategy. After consulting with Dr. Wes Warren (Washington University Genome Sequencing Center), we propose the following sequencing strategy. As with other recently sequenced mammalian genomes, a whole-genome shotgun sequencing strategy from a single individual will be implemented that incorporates paired-end reads from short, intermediate and large (fosmid and BAC) insert clones, with the expectation that the bulk of the sequence reads will be generated using 454 technology. As QTL mapping and population genetic studies are a planned avenue of future research in voles it will be highly desirable to generate a genome-wide panel of SNPs that could be used for those purposes. To augment the discovery of SNPs expected from the whole-genome shotgun reads used for the genome assembly, a light sampling of random reads (0.2X genome coverage) will be generated from a carefully selected group of seven additional, genetically diverse prairie voles and 2X genome coverage of a single meadow vole (*Microtus pennsylvanicus*). Finally, to aid in the accurate annotation of the vole genome and to support the development of microarrays, 10,000 ESTs will be sequenced from each of eight biologically relevant tissues with the 454 platform.

d. DNA source considerations. Like other rodents, prairie voles possess an XY sex determination system with males being the heterogametic sex. We are currently generating a 10X BAC library from a male prairie vole with a known pedigree. We propose to sequence a sister or female offspring (XX) of this male to maximize the ability to assemble the X chromosome. Targeted BAC-based sequencing could be used in the future to sequence the Y chromosome in this species. Access to the DNA from the primary individual used in the whole-genome shotgun as well as samples from other voles to be sequenced are readily obtainable from the Yerkes vole colony.

5. Are there other (partial) sources of funding available or being sought for this sequencing project? At this time, there are no other sources of funding available or being sought for this sequencing project.

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Index of Supporting Letters

UNIVERSITY OF MICHIGAN

Dear Larry,

It is tremendous news that efforts are under way to sequence the genome of the monogamous prairie vole. As our field is well aware, the robust expression of monogamous traits exhibited by this species has resulted in the prairie vole emerging as the premier animal model for studies of the neurobiology of social attachment. I have no doubt that having access to the prairie vole genome will enhance our understanding of adaptive and dysfunctional social behavior. In my lab, the prairie vole model provides a unique opportunity to examine the interactions between social behavior and drug addiction. Social environment is critical for drug abstinence in humans, however, understanding the neuroscience of the interactions between social and drug reward was very limited prior to application of the prairie vole model. Drugs of abuse take over brain circuits evolved to mediate adaptive social bonding and we have recently shown that the brains of 'pair bonded' voles are protected against the rewarding effects of abused drugs. This is due to the extraordinary adult neuroplasticity which occurs in this species following both social engagement and following drug intake. It would be of tremendous value to the addiction field to understand how social behavior can provide neuro-protection against drug reward. Important progress toward this endeavor will be made by comparison of the sequence and characteristics between human and prairie vole genomes. Further, compared to traditional laboratory rats and mice, prairie voles show extraordinary individual variation in many behavioral traits including their responses to drugs of abuse. Access to the prairie vole genome will facilitate our efforts to understand the genetics underlying susceptibility to drug reward over adaptive natural rewards. Improved understanding of the genetic susceptibility to drug addiction will hasten the identification of children that will be at high risk of drug abuse. Creation of transgenic prairie voles, insertion of prairie vole genes into mice, and other genetic technology, that are available at the Transgenic Animal Model Core at the University of Michigan, would be greatly facilitated by access to the prairie vole genome.

While my primary interest is focused on social and drug interactions, it must also be noted that access to the prairie vole genome will have enormous impact on the filed of social attachment. Additionally, with the rapid adaptations seen across vole species, sequencing the prairie vole genome may well be a landmark moment across the entire community of evolutionary biologists.

Sincerely,

Brandon J. Aragona

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LA JOLLA, CALIFORNIA 92093

La Jolla, July 2, 2008

Dear Larry,

This letter is in support of your white paper proposal for sequencing the genome of the prairie vole (*Microtus ochrogaster*).

In your proposal you suggest to obtain a high-quality 6X coverage draft assembly of the prairie vole genome. In addition, you propose to obtain a 2X coverage sequencing of a related asocial species, the meadow vole (*Microtus pennsylvanicus*), and the establishment of an EST sequence database. These resources will complement other genomic tools that are being developed for prairie voles and will ensure that this species maintains it current position as a powerful animal model for basic and translational behavioral research.

Prairie voles are a very powerful and well-established model species in behavioral neuroscience with discoveries made in prairie voles have led directly to novel treatment strategies for psychiatric disorders in humans. In addition, voles of the genus *Microtus* also serve as widely used model organisms in ecological and evolutionary research, since *Microtus* voles are found throughout the Northern Hemisphere and they are among the most abundant mammals in many habitats. They represent one of the best-studied groups of mammals with regards to their ecology, and display a number of features that make them ideal for studies of within-species diversification and speciation.

As you know, my lab is studying the creeping vole, *Microtus oregoni*, because of its unusual sex chromosome system. In most mammals, females have two X chromosomes, and males have an X and a Y chromosome (XX vs. XY). However, in *M. oregoni* males are XY, but females are X0 (i.e. females only have one X chromosome). Thus, the X chromosome in these species is completely sheltered from recombination. My lab is using population genetics and molecular evolution approaches, to investigating the evolutionary origin of this bizarre sex chromosome system, and to study the evolutionary consequences of a lack of recombination on the X chromosome of this species. A main obstacle we face studying *M. oregoni* is the current lack of genomic resources, and in particular genome sequence information, for this species group.

Thus, establishing genomic resources, including the genome sequence for a species of the genus *Microtus* and an EST database would be a great benefit for making progress in *Microtus* research in general, and would greatly help our own research on the evolution of sex chromosomes in *M. oregoni.*

Good luck with your proposal,

Sincerely,

Doris Bachtrog

UNIVERSITY OF CALIFORNIA, DAVIS

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ONE SHIELDS AVENUE
DAVIS, CALIFORNIA 95616-8686

Dear Larry,

I am writing this letter to express my enthusiasm for the proposed sequencing of the prairie vole genome. Over the last few decades, prairie voles have emerged as the primary model system for the evolution of mammalian social bonding. Given the rising incidence of disorders which affect social bonding, I am positive that having ready access to vole-specific genomic resources will lead to a landslide of new, biomedically relevant research.

In my own laboratory I study the long-term, developmental consequences of various types of early experience on the later ability of prairie voles to form a social bond. These can be natural manipulations (differences in parenting) or pharmacological manipulations that mimic human experience (exposure to oxytocin around the time of birth). The ability to examine the epigenetic consequences of these experiences, and the changes in gene expression associated with them, would be key to successfully connecting early experience to adult outcomes. These studies would be much facilitated by the sequencing of the prairie vole genome.

Finally, I have no doubt that the availability of the genomic sequence will draw many new scientists to the prairie vole research community, accelerating the use of this species as one of the premier animal models for understanding genetic and epigenetic bases of variation in social behavior. This will undoubtedly lead to new and exciting discoveries with direct relevance to human mental health and disease.

Sincerely,

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May 20, 2008

Dear Larry,

I am thrilled to learn that you and your colleagues are proposing to have the prairie vole genome sequenced. As you know my laboratory use the prairie vole model to understand the neurobiological mechanisms underlying parental care. I strongly support your proposal and fully agree with your objective of facilitating genomic research in vole model systems. Studies conducted by members of the vole research community have already established that prairie voles are ideal animal models for research in social behaviors. In particular, your work regarding intraspecific variations in repetitive DNA sequences for vasopressin receptor subtype (V1aR) of prairie voles shows promise in gaining a better understanding for the root causes of autism, a psychiatric disorder in which the V1aR gene has been implicated. I look forward to the day when we have access to the prairie vole's full genome sequence as that would greatly enhance my research in determining the mechanisms of epigenetic effects on parental bonding, as well as giving us a better understanding of the evolution of parental behavior.

I am confident that sequencing the prairie vole genome would not only benefit those of us who are interested in the neurobiology of social behaviors, but would also aid those who are determined to find a cure for psychiatric disorders such as autism. The availability of genome data will also help attract the next generation of molecular/genomics oriented researchers to this powerful model system, leading to important discoveries with direct relevance to psychiatry and mental health.

I wish you and your colleagues every success in your application.

Best Regards,

Maryam

Maryam Bamshad, Ph.D.
Assistant Professor
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CUNY

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SCHOOL OF MEDICINE

Department of Psychiatry
Center for Neurobiology and Behavior

June 12, 2008

Larry J. Young PhD
William P. Timmie Professor
Center for Behavioral Neuroscience
and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry,

I am writing to express my overwhelming support and excitement for your whitepaper to NHGRI to sequence the prairie vole genome and to develop other genomic resources. As you know, I am a mouse behavioral geneticist interested in identifying genes regulating mouse social behavior. As you know, we have already drawn from your work in the prairie vole and have data suggesting that polymorphisms the avpr1a receptor gene may contribute to strain differences vasopressin receptor expression and aggression. QTL analysis using NZB/B1NJ and A/J strains revealed that a segment of distal chromosome 10 is linked significantly with aggression. Based on yours and others work on prairie voles, we suspected the microsatellite polymorphism in the avpr1a gene, which lies within this segment, contributed to the behavior. Indeed, this seems to be the case. This example demonstrated that the prairie vole is a powerful animal model not only for studies related to human mental health, but also for mouse behavioral genetic studies. My laboratory also uses various mouse strains to model the behavioral phenotypes found in autism and schizophrenia. I strongly believe that prairie vole research will also inform our work in autism mouse models. For these reasons, I sincerely hope that we will soon have the prairie vole genome sequenced, which will greatly facilitate research in the prairie vole community, which I am confident will continue to inform my own mouse behavioral genetics research program.

Sincerely,

Edward S. Brodkin, M.D.
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C. S. Carter, Ph.D.
Professor of Psychiatry
Co-Director, The Brain-Body Center
E-mail: scarter@psych.uic.edu
May 12, 2008

Dr. Larry Young
Department of Psychiatry
Emory University
Atlanta, GA

Dear Larry,

I want to thank you and your colleagues for impressive efforts toward making the prairie vole a viable animal model in the study of genomic mechanisms of behavior. In addition, your success in finding support for the prairie vole genomic project will permit and encourage other scientists to bring new perspectives to the study of this unique animal model.

Prairie voles are readily reared and studied in the laboratory. In addition, ecologists have provided an exceptionally extensive natural history for prairie voles and related species, which supports the biological validity of studies done in the laboratory. Furthermore, the global presence of other arvicoline species with divergent and related traits provides natural experiments which strength the usefulness of this model in an eventual understanding the evolution of behavioral traits.

Even in the absence of contemporary molecular tools, research in prairie voles has provided breakthroughs in our understanding of the integrative neurobiology of social behavior and mental disorders that are characterized by dysfunctional social behaviors. Several novel traits of prairie voles that are shared with humans (but not found or less obvious in most laboratory rodents) include the capacity to form long-lasting selective social bonds, the appearance of spontaneous male parental behavior, alloparental behavior and the tendency to form extended families with incest avoidance.

Studies in prairie voles were the among first to identify the biological basis of sociality, implicating central neuropeptides including oxytocin, vasopressin and corticotropin-releasing factor in sociality in general and specifically in the formation of social bonds. These studies also have allowed the identification of previously unrecognized sex differences in the neurobiology of normal and atypical behaviors, including sociality and parenting. Studies in prairie voles have documented the capacity of developmental exposure to oxytocin and vasopressin to produce developmental changes in the mammalian nervous system, in part through re-programming in the expression of these peptides as well as their receptors. In addition, studies of prairie voles are providing a new model for the epigenetic effects of early experience and parent-offspring interactions, which also appear to be acting through long-lasting changes in peptidergic systems. Most recently, working in unrestrained animals, we have discovered that prairie voles have a novel human-like autonomic nervous system, with high levels of parasympathetic activity. Knowledge of the neurobiology of the autonomic nervous system allows us to understand the mechanisms through which social interactions and social support, possibly acting through peptidergic system, are neuroprotective in various human diseases including autism, schizophrenia, anxiety, depression and heart disease.

As you know scientists working with voles are currently hampered by the lack of knowledge of the full prairie vole genome, without which many important molecular methods are not available for use in this species. Adding prairie voles to the genome project will be a productive and natural extension of the larger aims of that work. You have my full support and best wishes for the success in this endeavor.

Warm wishes,

C Sue Carter

Professor and Co-Director

YERKES NATIONAL PRIMATE RESEARCH CENTER
Division of Neuroscience
EMORY

Larry J. Young PhD
William P. Timmie Professor
Center for Behavioral Neuroscience
and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry, May 23rd. 2008

I am writing to confirm that I am very excited about our collaborative effort to produce germ-line transgenic voles through the process of lenti-viral transfection of embryos. As you know I have extensive experience using this technique to produce transgenic animals including rodents and nonhuman primates. In 2001 I published a report in *Science* on the first transgenic rhesus monkey, and this month we published a report in *Nature* on the first transgenic monkey model of Huntington's disease. I am very happy that we have now been successful in transferring this technology to voles and have generated the first transgenic vole! I am very excited about your plan to use transgenics in combination with siRNA to investigate the role of specific genes in the regulation of behavior. I am also very supportive about your proposal to get the prairie vole genome sequenced. The availability of genome data, which can be useful in generating transgenes and siRNA sequences, along with transgenic technology will open up some very exciting possibilities for prairie vole research. I am looking forward to continued collaboration.

Sincerely yours,

Anthony W.S. Chan DVM Ph.D
Yerkes National Primate Research Center
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UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Institute for Genomic Biology
1206 West Gregory Ave., Room 2406
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Professor David F. Clayton, Ph.D.

July 8, 2008

Larry J. Young PhD, William P. Timmie Professor
Center for Behavioral Neuroscience & Department of Psychiatry
Yerkes National Primate Research Center
Emory University School of Medicine

Dear Larry:

I am pleased to write in enthusiastic support of your white paper proposal for sequencing the genome of the prairie vole (Microtus ochrogaster). There is absolutely no question that this a timely and compelling goal. Indeed, it is surprising to me that a genome sequence for the vole is not already in hand, given the unique and proven value of this species for understanding the genomic roots of social affiliation.

As the lead author on the successful white paper proposing the zebra finch genome for sequencing, I appreciate the process you are going through. I have read through your white paper and find it very exciting. The vole is indeed the preeminent model organism for understanding genomics and neurobiology of social behavior – it's the "poster child" the rest of us point to when we think about relating variation in complex behavioral phenotypes to specific gene differences. I anticipate explosive results from applying a sequenced genome to the study of individual variation in traits like social attachment, parental care and altruistic behavior as you have outlined.

I have no doubt that the resulting discoveries will have important implications for human mental health. In addition to its intrinsic value for the vole model, I also note that the genome sequence of Microtus will also help inform the genomics of the other major rodent model organisms in biomedical research (rats and mice). It will also nicely complement the research we are doing on songbirds as another model organism for study of gene-brain-behavior relationships.

It's an exciting time for research into the genetic and genomic underpinnings of social behavior. A genome sequence for the vole will make it even more exciting and productive.

Best wishes,

David F. Clayton, PhD
Professor of Cell & Developmental Biology, Neuroscience, and Bioengineering
Member, Institute for Genomic Biology and the Beckman Institute
Director, Songbird Neurogenomics Initiative: http://titan.biotec.uiuc.edu/songbird/
Co-Director, Songbird Genome Organization: http://songbirdgenome.org/

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Atlanta, GA 30322

June 5, 2008

Larry,

I am writing this letter to express my whole-hearted support for your proposal to sequence the prairie vole genome. Over the past twenty years, the prairie vole has become the premier animal model for studying the formation, maintenance, and expression of monogamous pair bonds in adult mammals. Using voles as a model system, we already have learned a tremendous amount regarding the neural substrates, endocrine influences, and behavioral modifications associated with pair bonding in mammals, but we have only scratched the surface in understanding the genetic bases for pair bonding. Given these strong theoretical and systems level bases, taking vole research to the molecular level is the next logical step, and likely will pay great dividends.

My NIH (HD48462, HD40722**)** funded research program is especially focused on studying the interactions between social attachment and reward processing and voles are the perfect model system in which to study such interactions. We recently have found that monogamous voles differ from promiscuous species in their responses to drugs of abuse such as amphetamine, suggesting the strong social bonds may reduce the addictive effects of such drugs. Having the sequence data from highly social voles will further such research by allowing comparisons to the human genome, and to those of species that do not display strong social ties.

Recent studies also have shown that prairie voles display autonomic responses that are more similar to those of humans than to other rodent species. Since human emotions are intimately tied to autonomic function, this finding further elevates the importance of the vole as an animal model for studying emotions *per se*, as well as disorders that target emotion/reward systems.

Finally, the fact that prairie voles share with humans the ability to express strong social bonds makes this species a potential gold-mine for understanding the causes of Autism Spectrum Disorders. In my opinion, even if there were no other benefits, this latter possibility alone makes sequencing of the prairie vole genome of critical importance since there are no other animal models for studying autism.

Thank you for undertaking this important project. You can count on my full support in your endeavors.

Good luck,

J. Thomas Curtis, Ph.D.
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The University of Akron

June 2, 2008

Larry J. Young, PhD
Center for Behavioral Neuroscience
Yerkes Primate Research Center
Emory University
Atlanta, GA 30322

Hi Larry,

I am writing with enthusiastic support of the Prairie Vole Genome Project. It is timely and important. Sequencing the vole genome is essential as "we" continue to develop and use the prairie vole model for translational biomedical research. The prairie vole with its human-like social system is rapidly becoming a primary rodent model system for the study of social behavior and mental health disorders associated with social deficits and quick access to genomic sequences will significant increase the value of this system. As you know we are studying the interaction of estrogen receptors and neuropeptides in regulating the expression of social behavior, and currently working with prairie voles in the study of depression and as a possible model for autism. The goals of the prairie vole genome project will provide many of the tools to help carry out this research. These resources will be of tremendous value to my lab and to vole researchers in general. We are very interested in genomic regulation and use viral vector mediate gene transfer to alter the expression of estrogen receptors (ER). I have received two grants from NIMH to support this work. The first, an R21, we increased ERα in the medial amygdala in male prairie voles which resulted in the predicted disruption of the expression of the high levels of prosocial behavior. The manuscript reporting these results was just accepted, with revisions, by the *Journal of Neuroscience*. The second grant, starts in July 2008 (NIMH), we will utilize RNAi to inhibit the expression of ERα and observe the effects on pair bond formation and alloparental behavior. Additionally, since we last spoke I received notification from NSF that they will be funding my grant to study the effects of early social environment, using prairie voles, on the expression of receptor patterns as related to social behavior and heritability of these patterns.

In your letter discussing the goals of the prairie vole genome project you lay out 12 ways in which this project will facilitate research using voles as a model system. I not only agree with all of them, but many are relevant to my current and planned research program, such as viral vector production, microarray analysis, qPCR, and comparative studies and evolutionary studies. Time and again we have faced the challenge of spending large amounts of time and effort to develop our own antibodies, i.e. it took us more than 2 years to produce the first successful prairie vole ERβ primary antibody, or probes for our studies. Successful sequencing of the prairie vole genome would greatly enhance our productive. This becomes even more critical as we continue to train researchers producing more labs that use prairie voles as their model system of choice. Good luck with your proposal and I look forward to taking advantage of the results.

Sincerely,

Dr. Bruce Cushing
Chair, Department of Biology
Director, Integrated Bioscience Program

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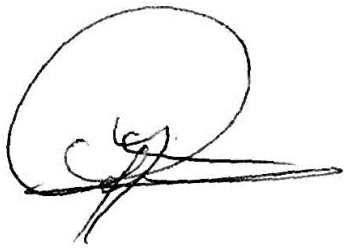
June 9, 2008

Dear Larry,

I am very enthusiastic about your plans to sequence the vole genome. This rodent provides us a wonderful opportunity to learn more about the neural basis of social behavior. As I am sure you know, we have found that mating and parenthood dramatically changes vasopressin expression in the projections of the bed nucleus of the stria terminalis and medial amygdala. As your work shows that the vasopressin receptors at the receiving end of these projections control the very behaviors that change after mating, studying this system will help us understand how the brain generates and modulates social behavior.

As the mating-induced changes in vasopressin expression appear to be long-term, they likely involve switches in the molecular machinery that regulates this system, possibly changes in the epigenome. Although the molecular control of vasopressin expression is relatively well understood in rats and mice, not much is known about this system in voles. Sequencing the vole genome would help us tremendously as it would allow us to formulate hypotheses that may explain how social experiences change the neural circuits that generate social behavior. Quite importantly, it would allow us to develop the molecular probes to test such hypotheses.

Sincerely,



Geert J. de Vries,
Professor

PURDUE
UNIVERSITY

DEPARTMENT OF FORESTRY
AND NATURAL RESOURCES

23 May 2008

Larry J. Young, Ph.D.
Center for Behavioral Neuroscience
Yerkes National Primate Research Center
Emory University
954 Gatewood Road NE
Atlanta, GA 30329

Dear Dr. Young,

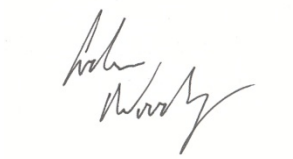
I write in strong support of your proposal to sequence the prairie vole genome. As you know, my lab has used voles to study two primary aspects of molecular evolution: the translocation of mitochondrial genes to the nuclear genome, and nucleotide substitution rates. In both regards, voles are remarkable.

Rodents are known to evolve more rapidly than most other mammals, but voles seem to be at the "tip of the spear". The genus *Microtus* contains more than 60 known species (and probably more that are undiscovered because of their phenotypic uniformity), all of which have evolved in the last ~1.2-2.0 million years. This rapid rate of speciation may be driven in part by extraordinary chromosome evolution. Robertsonian fission and fusion events throughout the genus have led to diploid numbers ranging from 2n=17 to 2n=64. The vole genome evolves rapidly not only at the chromosome level, but also at the DNA level. Our work has shown that rates of mitochondrial DNA (mtDNA) substitution are greatly elevated relative to other mammals, and that this elevated rate of nucleotide substitution extends to the nuclear genome.

In addition to substitutions, voles seem especially prone to nuclear insertions of mitochondrial sequences (numts). We have documented roughly as many numts in *Microtus* as in *Mus* or *Rattus*—despite the fact that those genomes are completely sequenced, and we have only ~27kb of vole sequence! This strongly suggests the vole genome contains scores more numts than other rodent genomes, but only the complete genome sequence will allow this determination to be made with certainty. It seems to me like these numts might be good models for gene therapy and their delivery systems. What is it about voles that allows them to tolerate such a high rate of gene insertion? Is it somehow related to chromosome fission and fusion events? And what about recombination? Are hotspots somehow tied to numt insertion, or to fission/fusion events? It is yet unclear how (or even if) these various facets of genome evolution are coupled, but a genome sequence would open entire new avenues of research.

A vole genome sequence would greatly accelerate my own research program, but I also believe the entire scientific community would benefit from a more detailed examination of genome evolution in these creatures who seem to evolve at unprecedented rates.

Sincerely,



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DEPARTMENT OF PSYCHOLOGY המחלקה לפסיכולוגיה

17-May-08

Dear Prof. Young,

Re: Vole Genome Consortium

I would like to offer my strong support for the Vole Genome Consortium and its goal of genotyping the prairie vole genome. My own studies in human behavioral genetics, and especially our molecular genetic studies of autism, have benefited tremendously from the ground-breaking investigations that initially focused on the prairie vole. Indeed the studies of the social behavior of the vole and the elucidation of the crucial role played by nonapeptides, vasopressin and oxytocin, has been an inspiration and extraordinarily informative for studies of human behavior and autism in particular.

We have shown that genetic variation in the promoter region of the arginine vasopressin 1a (AVPR1a) receptor contributes risk to autism primarily mediated by deficits in social communication, core deficits in this disorder(1). Remarkably, it is also the promoter repeat region in the vole which is so critical to its social phenotype. Again, based on knowledge gained in studies of the prairie vole, we were also prompted to examine the oxytocin receptor (OXTR) and demonstrated association between tagging SNPs across the OXTR gene and autism(2). Moreover, our studies of vasopressin and oxytocin are not limited to clinical disorders but we have extended our research to behavior in non-clinical groups. A seminal study by my group of the role of the AVPR1a receptor in facilitating altruistic or prosocial behavior, using an economic game paradigm, has recently been published(3).

We also have presented evidence that genetic variation in the AVPR1a receptor contributes to human personality traits such as perfectionism that may play a role in predisposition to eating disorders(4). Finally, we have explored the role of the AVPR1a receptor in human music(5, 6), a phenotype perhaps related to social communication which also has its antecedents in lower vertebrates. To summarize, in my research group translational research (reviewed in (7)) based on the vole has catalyzed an increasing number of studies regarding the neurochemical and neurogenetic basis for social communication in our own species. These considerations make me a strong advocate of the Vole Genome Consortium.

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10 May 2008

Larry J. Young, PhD
Center for Behavioral Neuroscience, Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry,

As you know, my research focuses on the social and sexual behavior of voles. I am also involved in the Tennessee Mouse Genome consortium as the PI for the social behavior domain. In that capacity, I was responsible for developing and conducting high-through put screens to identify mice that displayed phenotypic deviations in social and sexual behavior. This information was later used to determine the genetic underpinnings of social and sexual behavior. I strongly believe prairie voles represent an excellent model for understanding the neurobiological and genetic basis of social and sexual behavior. The natural history, behavior, physiology, and neurobiology of prairie voles are well documented in the literature. Thus from my perspective prairie voles represent an outstanding focal animal for such study. Moreover, their genetic and behavioral diversity make them an excellent model for discovering the role of genes in regulating social behavior. The development of genomic resources for prairie voles will allow researchers exploiting this model to the fullest. These tools will not only allow investigators to potentially identify genes contributing the behavioral diversity in prairie voles, but will also greatly enhance our abilities to generate clones that will be useful in molecular studies. I believe that it is important to add prairie voles to the list of species to have their genome sequenced. We think this will be a great benefit to the prairie vole research community

I applaud you on your effort to develop these resources for the prairie vole community.

Sincerely,

Dr. Michael H. Ferkin
Professor, Department of Biology
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JUNE 17, 2008

Professor Larry Young
William P. Timmie Professor
Center for Behavioral Neuroscience and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry,

I am writing in strong support of the proposal to sequence the genome of the prairie vole, *Microtus ochrogaster*. Over the past several years, research on the prairie vole, particularly the analysis of genetic influences on social behavior, has catapulted this model system to the forefront of mammalian species useful for understanding social interactions. The white paper describes clearly how many fundamental insights about the biology of social behavior as well as the applications to human health have resulted from these studies. Indeed, candidate genes whose functions have been elucidated in the vole studies to date have now been examined in many animal systems to good effect, showing that the discoveries in voles will have widespread value in the scientific community.

More broadly, while the insightful and careful work on the prairie vole has brought it to the forefront of scientific analysis, it is appropriate to note that over 40% of mammalian species are in the order *Rodentia* and these 2000 species have a staggering diversity in form and behavior. Rodents have successfully invaded nearly every continent and occupy almost every ecological niche. As such, the deeper understanding of the genetic underpinnings in voles could lead to insights in a wide range of social behaviors in other mammalian species. There are rodent species, for example, where large groups of animals are divided into smaller neighborhoods in which individuals baby-sit young, help construct each other's homes and cooperate along several dimensions. Indeed, the only eusocial mammal is a rodent, the naked mole rat. This means that sequencing the prairie vole genome will provide leverage into a large family of species with remarkably useful social behaviors open to analysis.

I believe that the sequencing of the prairie vole genome will not only offer novel insights into the remarkable social behavior of this animal but also in other important rodent species.

Sincerely,

Russell D. Fernald

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Department of Animal Biology

College of Liberal Arts and Sciences
515 Morrill Hall
505 South Goodwin Avenue
Urbana, IL 61801-3799

30 May 2008

Dr. Larry J. Young
Center for Behavioral Neuroscience
and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry:

I am writing in support of your application to The National Human Genome Research Institute to add the prairie vole, *Microtus ochrogaster*, to the list of animals to have its genome sequenced. Others can address the neuro-pharmacological-medical implications of having the genomic sequence available more to the point than can I. However, as the one who first predicted and then confirmed that the prairie vole displays behavioral monogamy, I can provide a somewhat philosophical rationale for sequencing the genome of the species.

The prairie vole "story" is a classic example what began as purely esoteric basic research and ended up with important human health applications. We tested the field observations of apparent behavioral monogamy in the prairie vole, a relatively obscure species of small mammal, because of the uniqueness of monogamy among mammals. There was no thought of any applied application of the results of first the field, then the laboratory observations and experimental tests to verify that the species displayed a behavioral monogamous mating system. It was after Dr. Sue Carter and her group began conducting research into the physiological mechanisms of the monogamous behavior, that the application of the neuro-hormonal system associated with the monogamous behavior to investigation of human neuro-behavioral problems became apparent. The prairie vole has now become a valuable experimental animal for studies of human neurological brain disorders. Thus, what started out as purely basic esoteric research on an obscure species now has major human mental health implications.

Sequencing the genome of the species, will move utility of the prairie vole to the next level of research into human mental health and psychiatric diseases. This not only will facilitate use of the prairie vole for human related research purposes, but perhaps will encourage others to continue research projects that initially appear to be purely basic research, with no human application. I therefore strongly support the request for adding the prairie vole to the species to have its genome sequenced.

Sincerely,

Lowell

Lowell L. Getz
Professor Emeritus
Ecology, Ethology, and Evolution

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UNIVERSITY OF ILLINOIS
AT CHICAGO

The Brain-Body Center
Department of Psychiatry
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Angela J. Grippo, Ph.D.
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May 12, 2008

Larry J. Young Ph.D.
William P. Timmie Professor
Center for Behavioral Neuroscience and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Dr. Young:

I am writing to offer my support for the sequencing of the prairie vole genome. As an experimental psychologist and behavioral neuroscientist, my current research program benefits greatly from employing this highly social rodent species. My research efforts with the prairie vole investigate the role of the social environment in mediating behavior, physiology, and brain function in the context of affective disorders (such as depression and anxiety) and cardiovascular disease. Access to the prairie vole genome will allow for identification of important genetic characteristics that interact with behavior, physiology, and the environment, and provide an opportunity for investigating more specifically the intersection of genetics, psychology, and neuroscience.

Whole genome sequencing of the prairie vole will offer an important benefit to my research program, as well as the research of many investigators such as those who employ this rodent model for the study of social behavior, reproduction, and comparative neurobiology. These efforts will increase our understanding of health and disease, and will have a significant impact on improving both mental and physical health of humans.

Thank you for undertaking this important initiative, and please let me know if I can be of further assistance to your efforts.

Sincerely,

Angela J. Grippo, Ph.D.
Department of Psychiatry and Brain-Body Center

UNIVERSITY OF LOUISIANA
MONROE

15 May 2008

Dear Larry,

Thank you for making me aware of your efforts to develop a genomics data base for the prairie vole (*Microtus ochrogaster*). Many behavioral ecologists, including myself, recognize the importance of integrating genomics into our work. The proposed database would facilitate this process, enhancing the quality of behavioral ecology research. Thus, I want to give my full endorsement of your project.

I am particularly interested in this database because the prairie vole is an exceptional model organism for studies on social and genetic mating systems. As you know, researchers initially showed that prairie voles are socially monogamous. This finding was critical because of the rarity of socially monogamous mammals other than humans. However, as a result of neurobiological and genomic work that you and others have conducted, we now understand that prairie voles are socially but not necessarily genetically monogamous. Moreover, growing evidence suggests that the prairie vole mating system may vary geographically. This understanding has obvious implications for evolutionary theory and conservation biology.

Numerous researchers, including myself, have studied the sociality and cooperative breeding of prairie voles. Our understanding of the fitness consequences of these behaviors (a foundation in behavioral ecology research) rests on our ability to efficiently develop and use microsatellite primers in field based studies. The availability of a common database such as the one that you are developing would greatly improve our ability to integrate microsatellite techniques into field studies, increasing our understanding of the evolutionary significance of prairie vole sociality.

I want to highlight that your project could have broad reaching application outside of the prairie vole research community. For example, the development of your database falls in line with new research that you and I are developing with colleagues at National Taiwan University. Like the prairie vole, the species we hope to study in Taiwan, *Microtus kikuchii*, is socially monogamous. Our ability to study the fitness consequences and genomics of this species depends on our collective ability to develop techniques and databases for the *Microtus* genus. Thus, the development of a genomic database for the prairie vole could increase our efficiency in studying *M. kikuchii* and lead to future comparative work between these socially monogamous species.

Finally, as a faculty member at a largely teaching focused university, I recognize the potential educational benefits of your proposed database. The availability of database such as the one you are proposing could open new avenues of student-focused research at smaller schools like the University of Louisiana at Monroe. This would certainly enhance the quality of science education in this country.

Sincerely,

Dr. Loren Donald Hayes
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June 6, 2008

Larry J. Young, Ph.D.
Center for Behavioral Neuroscience
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RE: Sequencing of the prairie vole genome

Dear Larry,

I enthusiastically support your initiative to sequence the genome of the prairie vole. As a vole researcher, it would be a great resource. For my research on the relationship between pelvic sensory signaling and maternal behavior, the prairie vole is the ideal rodent model due to the extreme importance of birth on the appearance of maternal behavior. With the sequenced vole genome, we will be able to more effectively identify hereditary and physiological contributors that promote the behavioral changes in female prairie voles at the time of delivery. Specifically, we are excited about the possibility of using microarray analysis to track changes in gene expression associated with changes in maternal behavior at the time of delivery. Such findings, in turn, will continue to shape my translational research looking at the effects of obstetric interventions (i.e., Caesarian delivery and epidurals) on postpartum mental health in women. Because of the clinical implications of research using prairie voles, it is important that we know as much as possible, including the genome, in order to expedite a quick translation into treatment and/or policy.

The availability of the resources, such as microarray capabilities, will open up many new opportunities in my own research program and will help attract genomics-capable students and postdocs to my laboratory. Moreover, with the increasing popularity of prairie voles in research, this is an ideal time to sequence the vole genome.

Sincerely,

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UNIVERSITÄT BERN

Zoologisches Institut

Computational and Molecular Population Genetics (CMPG)

Bern, June 6, 2007

Genome sequence of the prairie vole *Microtus ochrogaster*

Dear Larry,

It is a pleasure to write a letter in support of your exciting proposal to the U.S. National Human Genome Research Institute to sequence the genome of the prairie vole *Microtus ochrogaster*. As an evolutionary biologist with interests in molecular evolution, comparative genomics and speciation, I am at least as excited about the prairie vole genome as about the mouse or rat genomes. Obviously, there is the fantastic opportunity for comparative genomic analyses between voles and the other two rodents. We have initiated such analyses already in the past but missing information from voles has limited their scope strongly. A vole genome would instantaneously take all attempts at identifying genomic regions undergoing adaptive evolution in rodents to a much higher level. The phylogenetic position of voles in the Cricetidae as the sister family to the Muridae (mice and rats) will likely give an additional boost to the power of such evolutionary analyses.

The prairie vole genome will also open new possibilities for our research on why and how speciation occurs. Rodents comprise with almost 2300 species more than 40% of all mammals. *Microtus* is the most rapidly evolving mammal genus and comparative analysis of the genome of one or two species will definitely help to identify possible reasons for the extraordinary evolutionary success of rodents compared to other mammal and vertebrate orders. Access to genomic data from voles will enable us to search for differences in the architecture of genomes on the verge of speciation and it will give us access to specific genes and gene complexes involved in the speciation process. This is of particular importance since the recent evolutionary history of voles is not associated with that of humans, unlike mouse and rat, and therefore less disturbed. Of course, the prairie vole genome would also allow us to transfer and develop a wealth of molecular markers for other vole species that could be used to trace their evolutionary histories or investigate parentage and kin structures in natural populations. I know many colleagues who have been waiting for such a resource to be made available.

As you can see, a vole genome would be a remarkable resource not only for biomedical research but also for evolutionary biology. Access to a vole genome would bring these and potentially other scientific fields to a new level. This is a fantastic opportunity. I strongly support the proposal for the prairie vole genome and I very much hope that it will be sequenced very soon.

Sincerely,

Gerald Heckel

Dr. Gerald Heckel
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HARVARD UNIVERSITY
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Hopi E. Hoekstra
John L. Loeb Associate Professor of Biology

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July 05, 2008

Dear Drs. McGraw and Young:

I am writing in strong support of your proposal to sequence the complete genome of voles (genus *Microtus*). There are few species whose genome sequences will have such a direct utility for studying ecologically and biomedically relevant phenotypes as *Microtus*.

As you are well aware, *Microtus* has many advantages as a model system for the study of social behavior and translational medicine. First, they can be easily bred and maintained in the laboratory. Second, they are well-studied ecologically and have a range of interesting behaviors that can be studied both in the lab and in the field. Many of these behaviors are of interest both to evolutionary biologists as well as medical geneticists. Finally, and perhaps most-importantly, *Microtus* biologists already have generated a set of valuable experimental tools, including the use of viral vectors, siRNA and transgenics, which are essential for the functional tests of genes identified by microarray, QTL or comparative genomic analyses. Thus, it terms of becoming a "true" model system, *Microtus* is really only missing genomic tools.

As you know, our lab is studying adaptive morphology and behavior in deer mice (genus *Peromyscus*), and the genome sequencing of four *Peromyscus* species is currently underway. Having the genome sequence of *Microtus* also will be of great utility to the growing number of *Peromyscus* biologists for several reasons. First, because of close evolutionary relationship between *Peromyscus* and *Microtus* (both members of the subfamily Cricetinae), comparative analyses of gene and genome evolution will be greatly facilitated by the *Microtus* genome. This comparison of *Peromyscus* and *Microtus* is much more appropriate in terms of evolutionary distance than comparisons with either *Mus* or *Rattus* (subfamily Murinae) and will be especially useful for the identification of regulatory elements and rapidly evolving genes. Second, because *Peromyscus* also display similar -- although not as well-studied -- variation in social behavior, comparative genome analyses can be used to augment the search for genes or regulatory elements that affect social behaviors and social diseases. Our lab will benefit directly from the complete genome sequence of *Microtus*.

In conclusion, evolutionary and biomedical research that utilizes *Microtus* as a model system will undoubtedly greatly benefit from a complete genome sequence. This genome sequence will further enable the generation of additional genomic resources, including microarrays and SNPs. Moreover, the *Microtus* genome will be of utility to other researchers studying rodent biology.

Sincerely,

Hopi E. Hoekstra
John L. Loeb Associate Professor of Biology
Curator of Mammals, Museum of Comparative Zoology

HANS A. HOFMANN, PH.D.
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June 2, 2008

PROF. LARRY J. YOUNG
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AND DEPARTMENT OF PSYCHIATRY
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YERKES NATIONAL PRIMATE RESEARCH CENTER
EMORY UNIVERSITY SCHOOL OF MEDICINE
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Letter of Support

Dear Larry:

I am writing in support of the white paper you are preparing requesting whole genome sequencing for the prairie vole, which you will submit to NIH-NHGRI. As you know, I have been intimately involved in developing genomic resources for cichlid fish, culminating in the whole genome sequencing for four species currently under way at the Broad Institute, and thus are quite familiar with the potential impact such a resource can have on a model system community.

Obviously, there are few model systems in contemporary behavioral neuroscience that can compare to the vole system. As your own work in particular has shown, the prairie vole is THE mammalian model system for uncovering the molecular and genetic basis of complex social behavior with direct and important implications for human health. I expect that a draft genome and associated resources will very much enhance this fascinating research program, since you and your colleagues will be able to interrogate the entire genome in a much more straight-forward manner. Whether you want to profile the entire transcriptome or identify the genes (or SNPs) underlying a QTL of interest or find other microsatellites in promoters potentially associated with social affiliation and similar behaviors, all these approaches and many others will become more feasible. Given the rapid rise of next-generation sequencing technologies, it will become even possible to resequence numerous individuals and thus study the association between genomic variants and quantitative behavior across individuals. An added bonus will be that genomic resources will enable more detailed evolutionary studies into the origin of monogamy and other mating systems.

Given my experience with the process you have embarked upon and the incredible opportunities a vole genome will provide, I would be happy to provide input on your project as you are moving forward. I very much hope your proposal will be reviewed favorable and that you will succeed.

Best wishes,

MOUNT SINAI
SCHOOL OF
MEDICINE

Eric Hollander, M.D.
Esther and Joseph Klingenstein
Professor and Chair of Psychiatry

Director, Seaver and N.Y. Autism
Center of Excellence

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May 23, 2008

Larry J. Young PhD
William P. Timmie Professor
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Yerkes National Primate Research Center
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Atlanta, GA 30322

Dear Larry,

I am writing to express my enthusiastic support of your efforts to get the prairie vole genome sequenced. I have been following the prairie vole field for many years and your studies in voles have directly influenced my own research to identify treatment strategies for autism spectrum disorder (ASD). Among other symptoms, patients with ASD display deficits in the social cognitive domain. Drawing from your work and others in the prairie vole community showing that oxytocin facilitates social attachment, we conducted a study to determine whether oxytocin might enhance social cognitive function in ASD. Indeed the results were very promising, and we published this work in Biological Psychiatry in 2006. I am also very excited about our joint grant application in which we propose to perform parallel experiments in human subject in New York and in voles in Atlanta. The voles have already proven to have construct validity in the pursuit of pharmacological treatments for autism. I am confident that the voles have much more to offer in this regard and developing a genomic data base and all of the tools that are made possible with that will lead to more targets for potential treatment.

Best Regards,

Eric Hollander

THE UNIVERSITY OF MEMPHIS

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Larry Young, Ph.D.
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Atlanta, GA 30322

14 May 2008

Dear Larry,

I am writing to express my support for the application to The National Human Genome Research Institute to add prairie voles to the list of species slated for genome sequencing. The prairie vole has already been developed to a great extent as a model system. It is already a valuable model for addressing a wide range of questions - from the evolution of social behavior and mating systems in mammals to the neuroendocrine mechanisms and developmental processes underlying atypical or pathological social behavior. Through the efforts of many researchers, we have great deal of information on voles in the wild, their evolutionary relationships to other rodents and other mammalian species, behavior, physiology, development, neuroendocrinology, and neuroanatomy. Recently, through your work and that of others, we have begun to gain information on genetics of prairie voles and with that we can begin to take a functional molecular biology approach to understanding the biology of social mammals. The genetic work that has been done so far should be just a beginning. With more complete genetic information on the prairie vole, there is the potential for break throughs in many areas of research, including in basic research that can serve the basis for clinical treatments for human psychiatric illness.

If the prairie vole genome is sequenced, there are many specific molecular approaches that can be pursued at a much faster pace than is already being done. Already work using siRNA to silence specific genes has been fruitful in determining what brain regions regulate specific behaviors. This work has been possible though because a few labs have invested their time in sequencing a handful of genes. If the entire sequence were available, it would allow researchers to develop molecular probes for in situ hybridization and siRNA sequences for gene sequences more quickly and for a wide array of genes. Another exciting area of research that would move forward with leaps and bounds with the sequence in hand is research on the mechanisms of epigenetic effects on behavior.

The prairie vole has already been developed as an excellent model system for understanding the mechanisms of social behavior. Once the prairie vole genome is sequenced, it will certainly have a significant impact on our understanding of human behavior and mental health. For everyone's sake, I hope your effort is successful!

Best wishes,

Kristin Kramer

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To whom it may concern: June 30, 2008

It is my great pleasure to write in support of a proposal to conduct whole-genome sequencing on the prairie vole (*Microtus ochrogaster*). The prairie vole has been a model species in the study of almost all aspects of small mammal ecology. The extensive field experiments on the species in recent years have brought us deep understanding on how ecological features of the environment, including food and predation, affect population fluctuations, and how population parameters affect social organization, and vice versa. In the laboratory, recent breakthroughs have brought us exciting findings on how genetic and neuro-hormonal mechanisms affect mating and dispersal behaviors. With the co-development of all these related fields, we are truly entering a new era. We are seeing how molecular mechanisms affect population dynamics and even higher up processes, such as ecosystem functions.

It is exhilarating. Yet, we need further development in ecological genomics to see the details. The sequencing of the prairie vole genome would definitely advance such development. It would be an incredible boon not only to small mammal biologists, but to the ecological research community as a whole.

As you can see, the prairie vole genome would be a remarkable resource that the research community, particularly molecular ecologists, could access and exploit to bring ecological research to a new level. This is a fantastic opportunity and I very much hope that it gets funded and given a high priority.

Sincerely,

Y. Kirk Lin, Ph.D.
Assistant Professor
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National Taiwan University

Department of Veterans Affairs
Medical Center
3710 Southwest U.S. Veterans Hospital Road
Portland OR 97239

June 5, 2008

Larry J. Young, Ph.D.
William P. Timmie Professor
Center for Behavioral Neuroscience and
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Atlanta, GA 30322

RE: Prairie Vole Genome Project

Dear Larry,

I am writing to express my enthusiasm and support for sequencing of the prairie vole genome. As a neuroscientist with interests in substance abuse and depression, I am very excited by the prospect of being able to more comprehensively utilize the prairie vole for identifying mechanisms contributing to the pathogenesis of these psychiatric disorders. In order to advance our understanding of and ability to treat depression in humans, there is an urgent need for a better animal model to study this as well as other psychiatric illnesses—including, but not limited to alcohol use disorders.

Our lab currently uses prairie voles to investigate social, neurochemical and immunological factors leading to increased alcohol preference and consumption. Given the strong genetic component putatively contributing to alcohol abuse in humans, sequencing the prairie vole genome would significantly increase the utility and translational capacity of this rodent model of alcohol abuse.

Knowledge of the prairie vole genome would also be of great interest to my lab in order to better understand depressive disorders in the context of inflammatory diseases. Co-morbid depression is common not only in patients with substance use disorders, but also in patients with infectious diseases and other medical conditions. Increasingly, pro-inflammatory cytokines are thought to play a role in the development and maintenance of depressive symptoms in these patient populations. The prairie vole genome would thus be a valuable resource and allow the identification of genes contributing to the development of depressive-like behaviors in voles, such as decreased social interactions and decreased sucrose intake (*i.e*., an indicator of anhedonia). Specifically, I would be able to use vole-specific DNA microarray chips to target, for example, pro- or anti-inflammatory cytokine genes that are altered in voles exhibiting depressive behaviors. Subsequent gene manipulation experiments could be conducted to silence or disrupt genes important for the development of such depressive behaviors. From a

psychopharmacological perspective, this will also enable investigators to develop more specific therapies to treat depression.

I look forward to conducting future research projects using the prairie vole as a model to better investigate the genetic and social factors contributing to psychiatric disorders. I wish you all the best with this genome project. I am confident that my own lab will benefit tremendously from the development of this important resource.

Sincerely,

Jennifer M. Loftis, Ph.D.
Assistant Professor, Psychiatry
Research Assistant Professor, Behavioral Neuroscience
Oregon Health & Science University
Research Scientist, Portland VA Medical Center

MICHIGAN STATE
UNIVERSITY

June 9, 2008

Dear Larry,

I am writing to indicate my wholehearted support for the proposal to sequence the prairie vole genome. The past decade has seen the prairie vole become the pre-eminent rodent model for studying the biology underlying complex social behaviors. This is not surprising, as their social structure is more similar to many primates than it is to that of most rodents. Clearly, studying a highly affiliative rodent has numerous advantages over studying social behaviors in non-human primates. Having the entire prairie vole genome sequenced will surely launch work with this species into a new era, and provide impetus for more scientists interested in the evolution, neural mechanisms, and psychological/physiological implications of affiliation to consider adopting the prairie vole model for their work.

Work in my own lab will be greatly enhanced by this sequencing. We recently cloned the prairie vole aromatase and tyrosine hydroxylase genes. Knowing the sequences of these genes in prairie voles, rather than inferring the sequences based on these genes in rats and mice, would have facilitated the development of probes for our upcoming *in situ* hybridization work. Furthermore, we are currently examining whether individual differences in the number of dopaminerigic cells in the male prairie vole amygdala may be related to individual differences in their social behaviors. Having the full sequences of the tyrosine hydroxylase and other dopamine system-related genes would be an important tool that would help us explore how individual differences in dopamine system gene expression lead to individual differences in social behaviors.

This is an exciting proposal that would yield an invaluable resource to biologists working in a number of sub-fields. I hope the proposal is very positively received.

Sincerely,

Joseph S. Lonstein, Ph.D.
Associate Professor

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Jenae M. Neiderhiser, Ph.D., Liberal Arts Research Professor of Psychology
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Wednesday, June 4, 2008

Larry J. Young PhD
William P. Timmie Professor
Center for Behavioral Neuroscience & Department of Psychiatry
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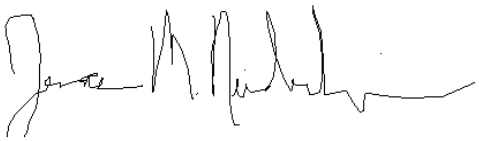
Dear Larry,

I am very pleased to hear that you are planning to submit a whitepaper to NHGRI to add the prairie vole to the list of species whose genome will be sequenced. As you know, my own work examines how genes and environment work together to influence various aspects of human relationships and subsequently on development and adjustment. I am fascinated by the prairie vole work and am deeply convinced that this unique animal model will make critical contributions to understanding the genetic mechanisms underlying parental-offspring relationship as well as pair bonding. Indeed, we have begun to explore whether the genes implicated in vole social bonding are associated with variation in human relationships using our own data and I am involved in a number of studies that have included genotyping of these genes because of the findings using voles. The development of genomic resources, such as high density SNP panels, EST arrays and high quality genome sequence in prairie voles will greatly facilitate the identification of novel genetic pathways that contribute to variation in social relationships in voles. I am confident that similar mechanisms will be identified in humans. I look forward to the exciting work coming from the prairie vole research community and plan to incorporate those finding in our human genetic studies.

Warm Regards,



Jenae M. Neiderhiser, Ph.D.
Liberal Arts Research Professor of Psychology
Professor of Human Development and Family Studies

12 JUNE 2008

Randy J. Nelson,
Distinguished Professor of
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Larry J. Young PhD
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Sent via email: lyoun03@emory.edu

Dear Larry,

Thank you and your colleagues for your impressive efforts to establish prairie voles as a viable animal model in the study of genomic mechanisms of behavior. Additionally, your success in finding support for the prairie vole genomic project will encourage other behavioral biologists to bring new perspectives to the study of this useful animal model.

We study seasonal changes in brain and behavior. *Mus* have been specifically bred to ignore environmental factors, and thus generally inappropriate for our NIH-funded and NSF-funded research on seasonality. Prairie voles are especially well suited for physiological investigations concerned with elucidating the neural and endocrine mediation of photoperiod on the brain, reproductive system, and behavior because they breed prolifically in captivity for many years and require routine laboratory care. Also, ecologists have provided an exceptionally extensive natural history for prairie voles and related species, which supports the biological validity of studies done in the laboratory. Generations of parental *Microtus* populations have been exposed to evolutionary pressures that produced the seasonal adaptations typical of this species. These pressures have sifted through the genotypic variation present in populations to generate the physiological adjustments that are central to our studies. For many proposed studies, only wild-caught or regularly outbred populations would be appropriate

Although prairie voles have been remarkably valuable in elucidating neurobiological mechanisms underlying social bonding, male parental behavior, and alloparenting, additional insights will be likely with modern genomic tools. We are particularly interested in the monomorphic traits between males and females of this species. Comparison of prairie voles which lack of sex differences in hippocampal volume, dendritic morphology, and spatial learning and memory performance with related species such as meadow voles will allow an understanding of basic mechanisms underlying adult neurogenesis, as well as

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seasonal changes in affective and cognitive functions. Having access to prairie vole genomic information will hasten progress in this regard.

As you well know, scientists working with voles are currently hampered by the lack of information regarding the full prairie vole genome, without which many important molecular methods are not available for use in this species. Adding prairie voles to the genome project will be a productive and natural extension of the larger aims of that program. In my opinion there are compelling reasons to conduct research on *Microtus*. Novel and important insights into biological mechanisms can be gained by using comparative methods. The increasing reliance on one or two model species could lead us to limited perspectives in biology and medicine. I hope that reviewers will appreciate the important niche that this model species fills and provide sufficient support to sustain the genomic efforts. Taken together, I am in full support of the Prairie Vole Genome Consortium great success and am excited about the opportunities it will make available to the prairie vole research community, as well as related research groups.

Cordially,

Randy J. Nelson,
Distinguished Professor of Social and Behavioral Sciences
Professor of Psychology, Neuroscience, and
Evolution, Ecology and Organismal Biology

RJN: jlb

UF UNIVERSITY of FLORIDA

Department of Zoology
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22 May 2008

Dear Dr. Young,

I am very pleased to write this letter in the strongest support of your NHGRI initiative to sequence the prairie vole genome. This is both an exceptionally valuable and timely project that will surely advance the state of several fields including evolutionary biology, behavioral ecology, molecular biology, and neuroscience.

As you are aware, I utilize the prairie vole model to study social behavior. Although the whole-genome sequences for the rat and mouse have been profoundly important, like most mammals their parenting habits and mating system is unlike those of humans. The prairie vole is arguably the best-studied non-human mammal similar to humans in these regards, and having a sequenced prairie vole genome means that researchers, such as myself, interested in understanding the neural mechanisms that influence monogamy, will be powerfully equipped to advance our current understanding of the mechanisms mediating social attachment and monogamous behaviors.

I imagine using a prairie vole genome sequence for my own work in the following ways:

- A genome sequence would facilitate the development of prairie vole microarrays, allowing for the identification of important genetic influences in neural regions known to be important in monogamous behavior (e.g. paternal care, or pairbonding).
- We can compare the structure and placement of genes that influence behaviors uniquely shared by humans and prairie voles, as well as the regions that regulate them. Any such structure-function relationships may reveal new insights into the genetic architecture of monogamy.
- The identification of new probes for in situ hybridization, will facilitate visualization of genes known to influence social attachment. Probes can also be developed for novel genes that influence such behaviors, as revealed by microarrays.
- Much of my research utilizes siRNA to silence gene expression of the vasopressin receptor and observe the consequences on social attachment. Other candidate genes have also been suggested to influence attachment and having access to the complete prairie vole genome sequence will facilitate the generation of new siRNA constructs and allow manipulation of new genes in isolation or combination.

The great potential to translate basic research done on the prairie vole to application, treatment and management of cognitive neuro-behavioral dysfunction in humans is exceptional. For this reason, the prairie vole is quickly becoming a traditional model for behavioral research and several new and exciting opportunities become available with a sequenced prairie vole genome. I am both personally encouraged and excited at the prospect of having this great resource available, and I believe this will prove to be among the most significant resources to advance understanding of human social behavior. I wish you every success in your application.

Sincerely,

Alex Ophir

UNIVERSITY OF FLORIDA

June 6, 2008

To whom it may concern:

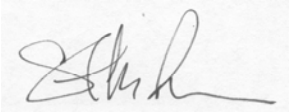
This letter is in strongest support of the proposal to sequence the prairie vole genome. The prairie vole has rapidly become a powerful for investigating the mechanisms of social behavior. Social cognition and its dysfunction are central to a diverse array of human health issues and intervention strategies. These include autism, schizophrenia, and the role of social factors in ameliorating stress-related pathologies such as heart disease and depression. A prairie vole genome sequence would help those of us interested in social cognition and its associated pathologies address core questions.

The work in my lab uses gene therapy vectors and RNA-interference to silence expression of individual genes in targeted brain regions of prairie voles. We are extending this approach to work in semi-natural enclosures, in which **we can genetically manipulate individual prairie voles and observe how individual genes interact with social environments to shape the behavior and development of other individuals**. Prairie voles allow us to explore the mechanisms of sociality in diverse genetic backgrounds and natural settings, providing an alternative and complementary approach to the examination of homogeneous lab mice in simple laboratory settings. Our work already demonstrates that allowing animals to interact freely in natural settings reveals results not predicted based on studies of conventional rodent models. (For example, we find natural variation in the cingulate cortex, an area implicated in schizophrenia as well as spatial memory, can be a good predictor of sociality and space use in natural settings. *PNAS* 105:1249-54.) A genome sequence would facilitate these efforts in several major ways.

- A genome sequence would make it easy to generate siRNA constructs targeting genes of interest.
- It would facilitate the development of new transgenics.
- A prairie vole genome could be combined with the sequences of *Mus*, *Rattus* and *Peromyscus* to facilitate the identification of non-coding regulatory motifs. Many important non-coding sequences are conserved within primates, but not between primates and rodents. This trend is thought to reflect evolutionary changes in transcription factor DNA-binding domains. A more complete sampling of Muroid rodents would permit identification of rodent regulatory sequences; this in turn would improve the ability of rodent models to guide more costly studies of primates.
- A genome would facilitate the use of sequencing-by-synthesis approaches to transcriptional profiling. This would obviate the need for the development of expression-based microarrays, and give a more complete picture of gene expression in targeted cells or brain regions.

Our lab anticipates using a prairie vole genome sequence in all of these ways. Such a resource would dramatically improve our ability to use this species to understand mechanisms of social behaviors and translate these results into therapies and interventions. Please feel free to contact me if you have any questions.

Sincerely,



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Madrid, June 9, 2008

Larry Young, Ph.D.
William P. Timmie Professor
Center for Behavioral Neuroscience and Department of Psychiatry
954 Gatewood Road
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry,

I would like to express my support for the proposal of the Prairie Vole Genome Consortium for the sequencing of its genome, which I think is of a great interest, not only for the scientific community, but also for human society considering the importance that the Prairie voles has as a model for the study of the neurobiology of social attachment and psychiatric disorders, such as autism and schizophrenia, as well as in research of neurogenesis and social- and drug-reward interaction and its underlying neuromechanisms.

The genetic sequencing together with the current progress that is being made in the field, as in the particular case of the interesting results that your group is obtaining, will permit great advancements. Like in the case of the project we are developing together, where the currently isolated genes or those that are to be sequenced, will be localized in their chromosomal location, shedding some light on the chromosomal rearrangement that might have had a role in behavior-related genes.

The studies performed with the Prairie Vole genome have provided results that prove that it is a species with great potential that should be deeply studied and hence, sequenced. There is a lack of knowledge about many

aspects of the genome, and that problem should be solved considering the great importance of the results that its study provides.

With the present letter I want to strengthen my full support for the Prairie Vole Genome Consortium.

Sincerely yours,

Miguel Pita
Associate Professor
Genetics Unit
Department of Biology
Universidad Autónoma de Madrid

UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

Swanlund Chair
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Urbana, IL 61801

1 July 2008

Larry J. Young PhD
William P. Timmie Professor
Center for Behavioral Neuroscience
and Department of Psychiatry
954 Gatewood Rd.
Yerkes National Primate Research Center
Emory University School of Medicine
Atlanta, GA 30322

Dear Larry:

I write to express my strongest support for your proposal to sequence the prarie vole genome. The prarie vole system has emerged as one of the most illuminating for the study of genes and social behavior, with landmark results emerging from the labs of Tom Insel, you, and others in the community over the years.

As you know, the study of vole social behavior actually has a long and distinguished history, but recent findings have elevated the profile of this model system to the stratosphere. These results include strong evidence for a between species differences in behavior and inter-individual differences within a species, differences in mating habits related to differences in brain wiring that relate to reward circuits, and provocative connections between vole behavioral variation and autism.

With a genome sequence in hand, I am confident that vole researchers will be able to provide a much-needed molecular bridge between pathological behavior and normal behavior. Your proposal is excellent, the vole genome is very much needed and eagerly anticipated, and I wish you the best of luck.

Sincerely yours,

Gene E. Robinson
Swanlund Chair
Professor of Entomology, Animal Biology and Cell & Developmental Biology
Leader, Genomics of Neural and Behavioral Plasticity Theme, Institute for Genomic Biology
Director, Neuroscience Program
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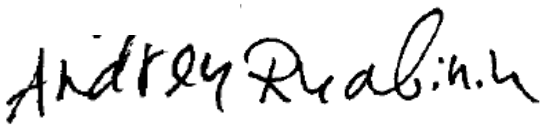
May 28, 2008

Dear Larry,

I was very excited to learn about the possibility of support from the The National Human Genome Research Institute for the sequencing of the prairie vole genome, and would like to express my fullhearted enthusiasm for this endeavor. As you know, my laboratory focuses on the mechanisms of anxiety and alcohol addiction. These behaviors and pathological conditions are strongly influenced by social and genetic factors, and have been extremely difficult to decipher because of lack of rodent models of human social behaviors. The recent development of prairie vole model holds a great promise for the understanding of human pathologies associated with social stress. In the prairie vole, for the first, time we have rodent model that exhibits high social interactions, social monogamy, and separation-induced depression-like behaviors. This species therefore, appears excellent for investigating the mechanisms of anxiety and alcohol-associated disorders induced by social stresses. Understanding these mechanisms is highly important for development for rational pharmacotherapies of these disorders. Having the prairie vole genome sequence would fuel mechanistic studies in this field, and could be the next important step towards development of these pharmacotherapies.

Sincerely,



Andrey Ryabinin, Ph.D.
Associate Professor
Behavioral Neuroscience
Oregon Health & Sciences University

02 July 2008

To:

Larry J. Young PhD

William P. Timmie Professor
Center for Behavioral Neuroscience and Department of Psychiatry
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Yerkes National Primate Research Center
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Atlanta, GA 30322

Phone: 404 727-8272
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From:

David H. Skuse MD FRCP FRCPsych FRCPCH

Professor of Behavioural and Brain Sciences
Behavioural and Brain Sciences Unit
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Telephone: +44 20 7831 0975
Fax: + 44 20 7831 7050 Behavioural and Brain Sciences Unit

Subject: The Vole Genome Consortium.

Dear Larry,

I was excited to hear that there are plans to map the genome of the prairie vole, and I am writing now to offer my unqualified support in this endeavour. We have been talking for some years about the importance of fully understanding the oxytocin-vasopressin systems and their regulation in voles, in order to uncover the basis of their influence on human social cognition. I am convinced from the data we already have, and the experimental work that is as yet unpublished, that these neuroendocrinological mechanisms really do have a critical role in maintaining mental health in humans. Dysregulation of those systems during early development can, I suspect, have seriously detrimental consequences for social adjustment leading to conditions such as autism.

For that reason, we are collaborating with a grant from NIMH, on a project to evaluate the role played by allelic variation in the human avpr1a receptor gene on risk of autism. It is clearly of enormous importance to understand in a model system the way in which these critical genes interact with others, to lead to variants of parenting and social behaviour, before moving on to experiments in humans. The Prairie vole offers an excellent example of such a model system and could offer so much more if we were able to clarify the genomic sequence and its naturally occurring variants.

At the present time, much work in the field of autistic disorders is atheoretical. There is much examining of genetic variants that may or may not have anything specific to do with social behaviour, rather than influencing cognitive abilities in general. In contrast, it seems to me, we would do better to support the exploitation of a well-worked out mechanism for the maintenance of social affiliation, which has increasing empirical support from a variety of species. The latter is a more rational use of limited funding. A first step would be the genomic sequencing you propose.

With kind regards,

David H. Skuse MD

MIAMI UNIVERSITY

DEPARTMENT OF ZOOLOGY

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June 17, 2008

Dr. Larry Young, Ph.D.
Center for Behavioral Neuroscience
954 Gatewood Road
Yerkes National Primate Research Center
Emory University
Atlanta, GA 30322

Dear Larry,

We are writing to express our enthusiasm and strong support for your proposal to be submitted to NIH to sequence the entire genome of the prairie vole, *Microtus ochrogaster*. Prairie voles have been an excellent experimental system for studying the behavioral and neuroendocrinological basis of social attachment and the formation of pair bonds in monogamous mammals. The entire genome sequence along with the other genetic tools that will be produced from this project will be a tremendous resource for our research as well as that of many others interested in the evolution of monogamy in mammals.

Specifically, we have been studying inter and intraspecific variation in social and genetic monogamy in geographically distinct populations of prairie voles as well as in voles living in semi-natural enclosures. We have been collecting behavioral and genetic data (parentage) annually from a natural population of voles in KS and another population in IN. Behavioral data includes information on the residency status of individuals (whether a resident at a single nest or a wanderer – individuals associated with multiple nest sites), composition of social units (male-female pairs, single males or females, or groups composed of more than two adults), space use and home range overlap with same sex and opposite sex conspecifics. In addition to the behavioral data, we have collected over 300 DNA samples from the KS population and approximately 800 DNA samples from the IN population that we are using for parentage analysis. At the end of the summer of 2008, we will have 3 years of data from both the KS and IN populations and we plan to monitor these populations for at least two more years. We have already found these populations to differ in several measures of social and genetic monogamy.

Recently we have begun to examine the extent to which genetic polymorphism among individual voles contributes to variation in social and genetic monogamy under ecologically relevant conditions. Currently only a handful of candidate genes (e.g. *avpr1a*) that may influence social behavior in prairie voles have been identified. Therefore, we are particularly excited about several of the genetic tools that will result form this proposal because we believe that they will have immediate benefit to our research in this area by enabling us to identify novel genes that are contributing to variation in prairie vole social behavior.

The high density SNP arrays that you propose to develop would enable us to screen the prairie vole genome to determine if particular chromosomal regions are correlated with measures of social and genetic monogamy in natural populations. If particular chromosomal segments are correlated with behavior, we can use the high quality sequence data to identify candidate genes within these regions. Finally, DNA microarrays would allow us to determine if differences in expression are correlated with the behavioral differences that we observe in field populations. Our archive of DNA samples from our field studies will permit us to look for correlations between specific genes and measures of social and genetic monogamy across hundreds of individuals.

Our studies of natural populations, as well as populations in enclosures, have shown us that prairie vole social behavior is influenced by environmental conditions (e.g. density, relatedness, habitat quality). Thus, while a great deal can be learned from laboratory studies of genes and behavior, we believe it is essential to explore the relationship between genes and behavior in an animal's natural setting. We are very excited about the genetic tools generated from your proposal, which will substantially enhance our endeavors in this area.

Sincerely,

Nancy Solomon

Nancy G. Solomon
Professor of Zoology and Director of the Center for Animal Behavior
Miami University
Oxford, OH

and

Brian Keane
Associate Professor of Zoology
Miami University
Hamilton, OH

Brian Keane

Louisiana State University
Department of Biological Sciences
202 Life Science Annex
Baton Rouge, LA 70803 USA
4 June 2008

I am writing to offer my strong support for the proposal to sequence the genome of the prairie vole (*Microtus ochrogaster*). The *Microtus* genome sequence would be a tremendous asset to many scientific disciplines including human health, behavioral ecology, mammalogy, comparative genomics, molecular evolution, population genetics and systematics. My dissertation research focused on the molecular evolution of voles, primarily within the genus *Microtus* and the genome sequence of the prairie vole would have greatly facilitated my work. In fact, when I was initially contacted about the efforts that were underway to conduct whole-genome sequencing on the prairie vole, I began to think of the more comprehensive studies that I would now be able to complete.

During my research, I examined rates of mitochondrial evolution within rodents and other mammalian taxa and characterized mitochondrial transfers within *Microtus*. The genus has a rapid rate of speciation and chromosomal evolution as compared to other mammals. I found that the mitochondrial substitution rate was accelerated in voles but was not able to effectively make conclusions about nuclear substitution rates. With a prairie vole genome sequence, we would be able to examine whether rates of evolution within the nuclear genome are concordantly elevated and whether rates of nuclear and mitochondrial evolution are coupled with rates of chromosomal evolution.

My work involving mitochondrial transfers to the nucleus would also be enhanced by a *Microtus* genome sequence. The transfers of mitochondrial fragments to the nucleus are thought to be facilitated by chromosomal repair mechanisms. Thus, *Microtus* voles, with rapid rates of chromosomal evolution, would be good models for the study of such transfers. I conducted a preliminary search for transfers and found that they seem to be more extensive within *Microtus* as compared to other rodents. However, I was only able to search a fraction of the genome. Because there have not been any defining trends as to the distributions of mitochondrial transfers within mammalian genomes, I could not extrapolate a genome-wide estimate. The sequencing of the prairie vole genome would allow for more sophisticated bioinformatic searches and would make a genome-wide estimate of mitochondrial transfers within *Microtus* possible. As a result, we could gain insight into the mechanisms behind the transfers of mitochondrial fragments to the nucleus.

A prairie vole genome would be a valuable resource and would bring about numerous opportunities for research. I sincerely hope that this proposal receives funding.

Sincerely,

Deborah Triant
Postdoctoral Research Associate
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Zuoxin Wang, Ph. D.
Professor

July 2, 2008

Larry Young, Ph.D.
William P. Timmie Professor
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and Department of Psychiatry
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Emory University School of Medicine
Atlanta, GA 30322

Dear Larry,

I am writing this letter to express my sincere and wholehearted support for the proposal of the Prairie Vole Genome Consortium. Prairie voles have been utilized as an elegant model for the study of the neurobiology of social attachment and psychiatric disorders involving disruptions in social behavior, such as autism and schizophrenia. In addition, prairie voles have recently become an important model for other phenomenon relevant to human health. For example, recently my lab has been making great progress in developing this animal model for the study of social- and drug-reward interaction and its underlying neuromechanisms. This animal has also been shown to be very useful for the study of adult neurogenesis, especially for the examination of the social environmental and endogenous factors that regulate cell birth and death as well as the functional significance of the newly proliferated cells in social behaviors.

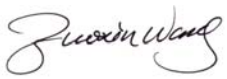
Although prairie voles are becoming widely accepted as an invaluable model for neuroscience studies with tremendous biomedical utility, investigators are currently limited in the number of tools available to fully take advantage of this unique system. The genomic resources that your group is developing would greatly enhance the research in the community that uses the vole model, as it will allow us to quickly isolate prairie vole specific sequences that we can then use as molecular probes. We would also be able to use these sequences to develop vole-specific siRNAs or viral vectors to manipulate gene expression. For example, in our current research project in collaboration with your lab, we use viral vector-mediated gene transfer to overexpress vasopressin V1a receptors in the anterior hypothalamus of the prairie vole brain to examine the role of these receptors in the regulation of pair bonding related behavior. Further, as you know, my lab has recently shown that in addition to displaying mating-induced pair bonding, prairie voles display conditioned place preferences to drugs of abuse such as amphetamine. Most interestingly, voles that are addicted to

amphetamine are less likely to develop mating-induced pair bonding whereas pair bonded voles need a high dose of amphetamine to induce conditioned place preferences, suggesting interactions between social- and drug-reward. This indicates a great potential for using prairie voles to study the neurobiological mechanisms underlying social- and drug-reward and as a foundation for the development of appropriate behavioral interventions for drugs of addiction. A full genome sequence would allow us to know details of the genomic context surrounding the target gene important for mediating social- and drug-reward interactions and to bring our studies to a much higher level of sophistication. Finally, the availability of these resources, and particularly the prairie vole genome, will also help us attract the strongest molecular- and genomics-oriented students who are currently biased toward mouse models because of the resources available to them.

Prairie voles provide a unique model for the aforementioned studies that traditional lab rodents are usually unable to offer. I strongly believe that with the rapid growth of the prairie vole research community and the availability of the genome sequence, the prairie vole will continue on its trajectory to become one of the preeminent biomedical models of human mental health with great advantages over other more conventional animal models.

I wish the Prairie Vole Genome Consortium great success and am excited about the opportunities it will make available to the prairie vole research community.

Sincerely,



Zuoxin Wang, Ph.D.