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REPORT ON THE LITERATURE

Regular readers of recent issues will notice two new items in the November issue of the Newsletter. These are brief reports of new developments in the literature, or themes drawn from these. . . . Such perspectives can be used to highlight not only important findings that may have attracted attention in some other forum, but also neglected findings, or those that may represent non-traditional approaches to neuroethology. For example, the two perspectives in this issue both underscore the importance of genetic determinants of behaviour, one by Troy Zars, on memory formation in *Drosophila*, and a second by Elizabeth Hammock and David King, on the relationship between microsatellite DNA and their role as mutationally adjustable regulators of animal behaviour.

GENES AND NEUROETHOLOGY: HOW CAN EVOLUTION ADJUST BEHAVIOR?

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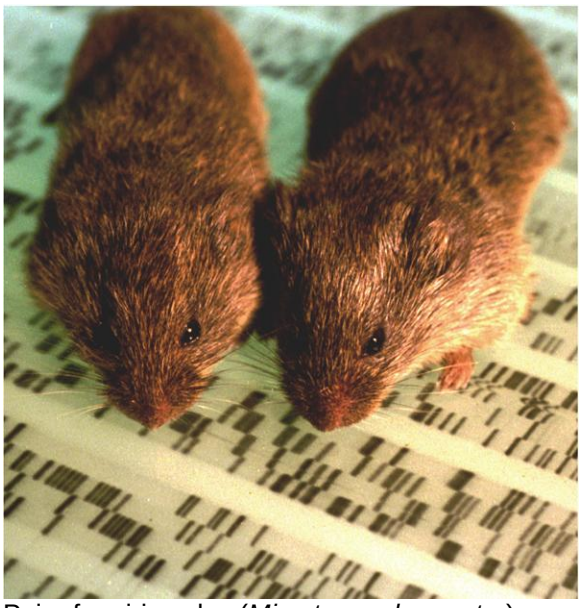
(This is the second installment in a continuing story. For part one, see *ISN Newsletter*, July 1997. http://www.neuroethology.org/newsletter/news_archive/isn.news.july97.sec2.htm#4.)

What genetic feature do the following phenomena have in common? (1) Neurotransmitter receptor distribution and associated social behavior vary among individual prairie voles (*Microtus orchrogaster*). (2) A gene influencing several aspects of *Drosophila* behavior has alleles whose frequencies vary with climate across several natural populations. (3) Several hereditary neurological and neuromuscular disorders display genetic "anticipation", such that children develop the disease at an earlier age, and with greater severity, than the affected parent.



Pair of prairie voles (*Microtus orchrogaster*)

Answer: In each case, the observed variation -- whether in social behavior, sensitivity to climate, or disease development -- depends on the number of motif repetitions in a tandem-repetitive DNA sequence (referred to here as microsatellite DNA). All tandem repeat tracts are susceptible to characteristic "slippage" mutations which incrementally increase or decrease the number of repeating motifs. These mutations occur at rates which may be several orders of magnitude higher than rates for single-nucleotide substitution. The resulting variation has proven to be immensely useful in forensics, pedigree analysis and marker-assisted selective breeding, to name a few applications. But the variation in microsatellites that provide such useful genetic markers has been widely regarded as "neutral", without any adaptively significant effect on phenotype (e.g., Ellegren, 2004). Until the early 1990s, hardly anyone expected microsatellites to have great functional importance.

Thus news of "repeat instability diseases" made quite a splash when, in rapid succession, Fragile-X, spinobulbar muscular atrophy, myotonic dystrophy, and Huntington's disease were each found to be caused by expansion of a tract of tandemly repeating base pair triplets. These disease-causing repeat loci were found both within and near coding regions of genes. At these loci, repeat number varies across healthy individuals. However, if the length of one of these repeat tracts exceeds a certain “pre-mutation” threshold (typically a few dozen repeats), then further mutations can cause extreme lengthening of the repeat tract, up to thousands of repeats. While there appears to be a length threshold for disease status, the length of the repeat tract above such a threshold can be associated with disease severity and age of onset. The susceptibility of expanded repeat tracts to further expansion underlies the clinical phenomenon of "anticipation", where successive generations have increased disease severity. Thus a child may inherit not just an abnormal parental allele but a freshlymutated, more-drastically-expanded version. The number of known repeat instability diseases has grown to more than forty; most are based on triplet repeat tracts, but some are also associated with repeating tetra- or penta-nucleotide motifs (Pearson, 2005). [Curiously, the human prion protein also contains an eight-amino-acid repeat, encoded by a 24 base-pair minisatellite motif. Of the 55 mutations known to be associated with Creutzfeldt Jakob disease, 27 involve the addition of up to nine additional 24-bp repeats (Leliveld *et al*. 2006).]

Certain features of the repeat instability diseases suggest questions of interest to neuroethology. First of all, the mutations which cause these disorders are not all-or nothing; the *number* of repeats matters, as shown by anticipation. Do these diseases reveal, in deleteriously exaggerated form, the existence of a previously unsuspected mechanism whereby the number of repeats in a *normal* microsatellite tract regulates some aspect of nervous system function? Second, since many examples known to date have profound impact on human nervous tissue, could repeat DNA hold some clue to the evolution of the human brain? Third, pathological repeat tracts are somatically unstable, so that variation accumulates in different parts of the diseased brain, even among non-dividing cells. Could normal microsatellites also generate somatic variation, perhaps contributing to neuronal differentiation in the developing brain? Finally, if microsatellites matter to the human brain, might they also have some broader significance for the nervous system and behavior of other animals?

The variation which arises from microsatellite mutation is so abundant that any given gene, in any given genome, is likely to be associated with one or more variable repeat loci. Microsatellites are commonly presumed to be "junk" -- after all, highly mutable genetic "stutters" seem unlikely to convey any reliable, or even useful information -- nevertheless evidence for a functional role for microsatellite variation has been accumulating for over two

decades. Not only are microsatellite mutations both frequent and reversible, but effects of repeat-number variation have been found for microsatellites located in exons, in introns, and in upstream and downstream regulatory domains. The number of repeats in a microsatellite sequence (and in minisatellites as well; the defining difference lies in the length of the motif) can influence practically any aspect of genetic function, from protein coding to exon splicing to regulatory interaction (Kashi & King, 2006).

So, what about those fruit flies and prairie voles? Evidence for the functional influence of microsatellite repeat number has come from many different studies involving many different organisms. But the most complete stories, which tie the effects of repeat number not only to measurements of gene function but also to the phenotype of intact, behaving animals, are those involving the *period* gene of *Drosophila*, which is involved in the regulation of the fly's circadian rhythm, and the *avpr1a* gene of mammals, which encodes a vasopressin receptor.

The fruit fly story was the first to emerge. Briefly, the *period* gene includes a hexanucleotide repeat, encoding a sequence of alternating threonine-glycine repeats. Variation in the number of repeats not only changes the length of this *thr-gly* run but also influences the temperature sensitivity of flies' circadian rhythm, and this variation apparently matters to flies living in natural environments. Several different repeat-number alleles occur in wild populations, and the frequency distributions of the more common alleles display a latitudinal cline. The shorter allele, which at warm temperature yields a circadian period closer to 24 hours, predominates in warmer regions, while the longer variant, which yields better temperature compensation so that temperature fluctuations have a lesser impact on circadian cycle, is more prevalent in cooler climates. This pattern, first reported for populations across Europe and north Africa, has recently been found in Australia as well (Sawyer *et al*., 2006). The frequencies of these repeat number alleles are even differentiated in populations separated by only a few hundred meters, across the sunny southfacing and shady north-facing slopes of "Evolution Canyon" in Israel (Zamorzaeva *et al*., 2005). Evidently, spontaneous repeat-number variation permits natural selection to "tune" the *period* gene to suit the local climate.

The vole microsatellite story has emerged over the past several years. Comparative analyses of closely related vole species of the neural mechanisms underlying social attachment, has demonstrated that the distribution of vasopressin receptors is a functional link in a chain that ties variation in brain activity to individual and interspecies differences in affiliative behavior and pair-bond formation. The junior author of this ISN news article (EADH) was privileged to join Larry Young's lab at Emory University as a graduate student at about the time that a remarkable species difference had been identified in the length of a complex microsatellite in the upstream regulatory domain of *avpr1a*, a gene which encodes one of the vasopressin receptor proteins (Young et al, 1999). Two monogamous species of voles (prairie and pine) have a very large microsatellite at this locus, while two non-monogamous species (montane and meadow) have a very small microsatellite locus. The prairie/pine locus is an order of magnitude larger than the montane/meadow locus. Knowing from prior literature that repeat number could have functional effects, members of the lab (especially me, Larry and his first post-doctoral fellow, Steve Phelps) were intrigued by the potential for this variation in microsatellite length to regulate the observed species differences in brain vasopressin 1a receptor distribution patterns and potentially species differences in behavior. I (EADH) was the lucky graduate student who got to investigate whether or not such a relationship existed. Using a series of approaches (cell culture, selective breeding, behavior and neuroanatomy) within and across closely related vole species, our results indicate

that repeat number in this microsatellite does indeed influence both the brain distribution of the vasopressin receptor and also the behavior of the voles (Hammock and Young, 2005).

If microsatellite variation matters for the behavior of individual voles, and if homologous microsatellites vary significantly among species, then perhaps microsatellites are one component in a general-purpose genetic toolbox for facilitating evolution. One metaphor for the role of microsatellites is that of mutationally adjustable "tuning knobs" (King *et al*., 1997).

Tuning knobs from the world of music.

When incorporated as functional elements into extended genes, tandem repeats provide a reliable and abundant supply of variation for efficient evolutionary adjustment of quantitative traits. Some microsatellites also provide reversible on/off switches for gene expression, a mechanism exploited for antigen switching by pathogenic bacteria (Bayliss, 2006) and also noted as the developmental-genetic basis for black spotting in red pigs, through somatic mutation (Kijas, 2001). Implications for neurobiology have barely begun to be explored, but tandem repeat variation has already been implicated in several aspects of behavior in humans and other primates. For example, increased susceptibility to stress-induced depression in humans (Caspi *et al*., 2003) and younger age at the time of a male rhesus monkey's dispersal from its natal group (Trefilov *et al*., 2000) are both associated with the shorter of two alleles of a repeat locus in the promoter of a serotonin transporter gene. This shorter allele reduces transcriptional efficiency of the serotonin transporter.

It may be important to clarify that we are not suggesting that microsatellite variation in genes is a privilege of genes involved in nervous systems. In fact, there is an irresistible example of microsatellites potentially involved in craniofacial development. Fondon and Garner (2004) compared microsatellite variation in the coding regions of genes known to be involved in craniofacial development across various dog breeds: changes in jaw morphology of breed standards over the past 150 years were associated with microsatellite length of those genes for craniofacial development. This rapid change in craniofacial morphology invokes images of Charles II of the Spanish Hapsburg family line of 17th century Europe. The Hapsburg royal family line contained many examples of what appears to be mandibular prognathism. Was the historical worsening (i.e. anticipation) of the "Hapsburg jaw" (and perhaps Charles' other disabilities) due to expanding repeats in genes with a role in craniofacial (and brain) development? Does consanguinity exacerbate anticipation?

Thus far, the data on the functional roles of microsatellites in inter- and intra-specific trait variation excites the imagination and raises many more questions. How generalizable are these findings? Are there certain gene ontologies that make the best use of such a mechanism? Are

certain taxa better positioned to take advantage of such heritable mutation? Have any genomes evolved mechanisms to regulate the rate of mutation at microsatellite loci (e.g. similar to mechanisms implicated in some cancers or akin to the "SOS" response in bacteria)? Do certain cell types (perhaps in the brain?) actively regulate somatic expansion and contraction of microsatellite loci? These questions are readily addressed with the plethora of molecular tools that can be used across taxa.

As evidenced from the robust diversity of heritable behavioral traits among closely related species, animal behavior evolves with great facility, and such rapid evolutionary adaptation must depend on novel non-lethal genetic variation. If we shift metaphors in our attempt to capture the genome-wide impact of variable microsatellite loci, we might imagine that each site paints a restless, shimmering pixel on a "molecular canvas", one in which an image of adaptive behavior is continually adjusted by the variation that microsatellite mutability provides.

Further reading:

Bayliss, C.D., and Moxon, E.R. (2006) Repeats and variation in pathogen selection. In: Caporale, L.H., ed. *The Implicit Genome*. Oxford University Press, Oxford, pp. 54-76. **An example of adaptive advantage conferred by variable repeats.**

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., and Poulton, R. (2003) Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science* **301**: 386-389.

Ellegren, H. (2004) Microsatellites: Simple sequences with complex evolution. *Nature Reviews Genetics* **5**:435-445. **An overview of microsatellites and their mutations.**

Fondon JW III, Garner HR. 2004. Molecular origins of rapid and continuous morphological evolution. *Proc Natl Acad Sci USA* **101**: 18058–63.

Hammock, E.A.D., and Young, L.J. (2005) Microsatellite instability generates diversity in brain and sociobehavioral traits. *Science* **308**: 1630-1634. **Describes the role of repeats in vole reproductive behavior.**

Kashi, Y., and King, D.G. (2006) Simple sequence repeats as advantageous mutators in evolution. *Trends in Genetics* **22**: 253-259. **Provides access to literature on functional effects of repeat-number variation.**

Kijas, J. M. H., Moller, M., Plastow, G., and Andersson, L. (2001) A frameshift mutation in *MC1R* and a high frequency of somatic reversions cause black spotting in pigs. *Genetics* **158**: 779-785. **An example of phenotypic effects resulting from somatic mutation in atandem repeat.**

King, D.G., Soller, M., and Kashi, Y. (1997) Evolutionary Tuning Knobs. *Endeavour* **21**: 36-40. **Introduces the metaphor of repeats as adaptively useful adjusters of gene function.**

King, D.G., and Soller, M. (1999) Variation and fidelity: The evolution of simple sequence repeats as functional elements in adjustable genes. In S. P. Wasser, ed. *Evolutionary Theory and Processes: Modern Perspectives.* Kluwer Academic Publishers, Dordrecht, pp. 65-82. **Develops the hypothesis that genes with adjustable repeats may be shaped by indirect selection.**

Leliveld, S.R., Dame, R.T., Wuite, G.J.L., Stitz, L., and Korth, C. (2006) The expanded octarepeat domain selectively binds prions and disrupts homomeric prion protein interactions. *Journal of Biological Chemistry* **281**:3268-3275.

Pearson, C. E., Edamura, K.N., and Cleary, J.D. (2005) Repeat instability: Mechanisms of dynamic mutations. *Nature Reviews Genetics* **6**: 729-742. **Reviews research related to the repeat instability diseases.** (This issue of NRG includes several other articles onrelated topics.)

Sawyer, L.A., *et al*. 2006 The *period* gene Thr-Gly polymorphism in Australian and African *Drosophila melanogaster* populations: Implications for selection. *Genetics* **174**: 465-480. **Latest instalment in the story of repeat variation in the *period* gene in *Drosophila*, with review of prior work.**

Trefilov, A., Berard, J., Krawczak, M., and Schmidtke, J. (2000) Natal dispersal in rhesus macaques is related to serotonin transporter gene promoter variation. *Behavior Genetics* **30**: 295-301.

Young, L.J., Nilsen, R., Waymire, K.G., MacGregor, G.R., Insel, T.R., 1999. Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. *Nature* 400 (6746), 766–768.

Zamorzaeva, I., Rashkovetsky, E., Nevo, E., and Korol, A. 2005 Sequence polymorphism of candidate behavioural genes in *Drosophila melanogaster* flies from 'Evolution Canyon'. *Molecular Ecology* **1 4**: 3235–3245. ***Period* gene frequency divergence in relation to local microclimate.**

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