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Dead Romanovs identified by PCR

Apart from the usual crop of gene assignments (for hereditary haemorrhagic telanglectasia, for example) this month's issue carries further the genetics of expanding repeating elements.

THE use of DNA analysis for forensic purposes still resembles laboratory investigation in that those responsible cannot behave as automata. So much is clear from the genetic identification of the remains, found in a shallow grave 35 km west of Ekaterinburg two years ago, of Tsar Nicholas II, his wife Alexandra and three of their children, reported in the current issue of *Nature Genetics* by Peter Gill *et al.* (**6**, 130; 1994). Most of those concerned are from the British Government Forensic Science Service at Aldermaston, but Pavel L. Ivanov, from the Engelhardt Institute in Moscow, and Erika Hagelberg, from the department of biological anthropology at the University of Cambridge, have played important roles.

Whether it is called an execution or a murder, the killing of Nicholas II and his family is one of the haunting tales of the Russian Revolution. Historians have long since established that the parents, at least three of the five children, three servants and the family doctor were killed on 16 July 1918 by soldiers of the Bolshevik army. The plan that the bodies should be disposed of down a nearby mineshaft was frustrated by the breakdown of the truck in which they were being carried; instead, they were buried in a roadside grave. Russian forensic investigations of the skeletal remains have tentatively established that the grave did indeed contain the remains of the Tsar and much of his family. Their positive identification is an important, if macabre, extra.

How do you set out to identify skeletons that have been buried in the ground for three-quarters of a century? Best start with bone. One gram or thereabouts will yield 50 picograms of DNA, enough (if only just) for the purposes of PCR. Then do you analyse the chromosomal or the mitochondrial DNA? The former is less stable over time, the latter more plentiful and maternally inherited, and so more indicative of relatedness. In practice, you have no choice but to go for both. Gill *et al.* worked with five different short tandem repeats in the nucleotide sequence of the genome proper, and with two well-known hypervariable regions of human mitochondrial DNA. Sex determination of the bones has been done by amplification of the gene amelogenin that is common to the X and Y chromosomes.

Prince Philip, Duke of Edinburgh and husband of the present British Queen, is the great-grandson, by maternal lineage, of the Tsarina's mother. Piquantly, a blood sample provided by the Duke is a crucial element in the identification of three of the skeletons at Ekaterinburg as those of sisters, and of Alexandra as their mother; all the mitochondrial sequences are identical. Ethicists will be intrigued that one of the first genetic sequences of a named and living individual to be published should be that of a member of a royal family.

The data also show that four of the nine skeletons in the grave at Ekaterinburg are not related to the Romanovs. They, presumably, are those of the doctor and the three servants. It follows that two of the children are missing from the grave. Gill *et al.* laconically note that the two concerned, the Tsarevitch, Alexei and his sister Anastasia, may have been burned or buried separately or have escaped.

The relationship between Nicholas II and his children is established by the similarity of the chromosomal DNA, which appears to show that the three children in the grave are the offspring of their parents, but Gill *et al.* stumble on an unexpected snag. The mitochondrial DNA from the Tsar's femur appears not to be chemically homogenous. Instead, for every mitochondrial DNA molecule with the base thymine (T) at position 16, 169 (in the standard notation), there are four molecules with cytosine (C) at the same place. This phenomenon, called "heteroplasmy", is a sign of recent mutation in one of possibly many mitochondrial DNA molecules. Plainly it is a matter of some importance for the forensic application of DNA analysis that such sources of confusion should be quickly recognized.

That, in a wider context, is one purpose of the article by Alec J. Jeffreys and five colleagues from the University of Leicester in the same issue (**6**, 136; 1994). They are concerned with the process of mutation in short tandem repeats, otherwise known as "minisatellites", in the human genome. One of these, known as MS32 (located on chromosome 1), consists of a stretch of DNA 29 base-pairs long that may be repeated between 12 and 800 times in different individuals, who will usually be endowed with minisatellites of different length by their two parents.

What determines the number of repeating units in a minisatellite? The question has an obvious bearing on the topical and even urgent question of the recently discovered role of repetitive trinucleotide sequences in several heritable neurological diseases, where severity and/or the age of onset is determined by the number of repeating units. What Jeffreys *et al.* now find (by the analysis of sperm DNA) is that mutation seems to accompany meiosis, that addition of repetitive units is more common than subtraction, that the 3' end of a minisatellite is more liable to mutation than the other and that the mutation rate, while always relatively high, seems to vary between individuals.

What this implies for the origin of the genetic diseases based on mutations of the repetition number is not yet clear, but Robert I. Richards and Grant R. Sutherland have a model (**6**, 114; 1994). The idea is that the expansion of a triplet (or other repeat) occurs during DNA replication. If there is a break in the newly synthesized strand, the loose end may jump to mate with another complementary trinucleotide, whereupon DNA repair enzymes will lengthen the repetitive structure. For a structure that is already long, that chance that there will be two breaks within the repetitive element will be greater, whereupon catastrophic lengthening is more likely, which fits in with the way in which the observed mutation rate increases with length.

Expanded repetitive elements are also now known to occur in the somatic cells of certain tumours, colorectal cancer in particular. Stimulated by those observations and the knowledge that the expansion of a CAG repeat is associated with Kennedy's disease, R. Wooster *et al.* (**6**, 152; 1994) have surveyed a dozen known repetitive elements in the human genome, measuring the two alleles in normal and cancerous tissue.

The upshot of the survey is unspectacular but none the less interesting. Alleles expanded relative to those in normal tissue were found in only 16 of 196 patients. Only one case of an expanded dinucleotide repeat was found (in a breast cancer), expanded trinucleotide and tetranucleotide elements accounted for the others. In no case was more than one repetitive unit expanded and more than one allele (paternal or maternal) affected. What that implies is that the circumstances in colorectal cancer, (or at least the non-hereditary form of it) must be exceptional. And may the sprinkling of cases found by Wooster *et al.* imply that expanded elements are but symptoms, not causes, of the general run of cancers? ®