Cross-examinations: DNA fingerprinting conference traces an impressive record

All plants and animals (including man) have far more DNA than they need to encode their genetic programs. Much of the excess is comprised of repetitious DNA with characteristic nucleotide sequences, repeat lengths and copy numbers. A high degree of length variation is often (but not always) seen when the repeats are tandemly arrayed, presumably because recombination between mismatched repeats generates copy number diversity in the resultant arrays. 'Alleles' of such highly polymorphic variable number tandem repeat (VNTR) loci, also known as minisatellites, can be distinguished either by PCR amplification using flanking primers or by restriction fragment length polymorphisms (RFLPs) using cloned repeat sequences as hybridization probes. Indeed, many minisatellites were identified first via PCR with random oligonucleotide (~ 10-mer) primers. DNA fingerprinting, i.e. the typing of polymorphic loci haplotypes, can distinguish between DNA from different sources and also be used to quantitate the genetic relatedness between individuals or even populations and species. The applications and implications of this deceptively simple technology were discussed by a wide spectrum of scientists, forensic experts, wild-life conservators and even some police officials who had gathered in Hyderabad for the recently concluded (December 13–16, 1994) Third International Conference on DNA Fingerprinting.

About a third of the presentations (including most of the plenary lectures) were related to the use of DNA fingerprinting evidence in forensics and personal identification. Of the two basic fingerprinting strategies, RFLP typing of VNTR loci and PCR amplification of short tandem repeat loci (STR, 1–6 bp repeat length), the first is more informative for identity testing, such as in cases of disputed paternity, where high molecular weight DNA is not limiting. The second strategy allows analysis even of degraded samples such as DNA from exhumed tissues and blood stains and is also much more sensitive. Many examples were given in which PCR analysis was shown to detect DNA from even a single hair or from cells deposited by a smoker onto a cigarette butt (Budowle), or specks of blood picked off a suspect's hand with cellotape (Petricevic). de Pancorbo even described the PCR analysis of DNA from the spongy bones of the 700–800 years old remains of a Basque individual.

Statistical methods to compare the data from the scene-of-crime DNA with suspects' samples (Newton Morton, Partha Majumdar) highlighted concerns about the abilities of juries (as in British and American courts) and even judges to cope with quantitatively presented evidence (John Brookfield, Ranajit Chakraborty). An example of the verbal mangling of logic is the 'Prosecutor's fallacy', which in its simplest form incorrectly equates the statements 'If A then B' and 'If not-A then not-B'. The need for compiling allele frequency databases of STR and VNTR loci for real populations was stressed by Newton Morton, Peter Gill and Ranajit Chakraborty. Only then is it possible to estimate the likelihood that a DNA fingerprint pattern obtained for the scene-of-crime sample matches that of a person chosen randomly from the subpopulation which includes the suspect. In this context, there was severe criticism of the 'ceiling principle', which somewhat arbitrarily allows the use of allele frequencies from different populations, even ones to which the suspect is not likely to belong. (A discussion of the 'ceiling principle' and other aspects of the reliability of forensic laboratory results, though important, is beyond the scope of this report.) Examples of database compilations came from Britain (Peter Gill) and France (Pfitzinger). Database compilations have also been attempted in India (Kashyap, Raina, Shuba Krishna and Karutha Pandyan), but these attempts need to incorporate the more recently developed DNA fingerprinting technologies and data management systems. One example of these new developments is the tagging of DNA with fluorescent dyes, which enables molecular size markers and the sample DNA to be loaded into the same lane of an agarose gel, or alternatively, it allows the typing of several PCR markers (polymarkers) simultaneously as in a multiplex system (Gill, Robertson). Another example is the use of two-dimensional (2D) separation of DNA fragments. Nurnberg used 2D fingerprints to compare DNA from tumour and normal tissues. A third example is the development of an alternative to gel-based analysis of DNA polymorphisms, that uses arrays of target DNA sequences printed onto either a glass or a plastic matrix. Hybridization to such arrays would allow digital read-out of the results (Southern).

A phylogeny reconstructed from allele frequencies of 13 STRs from 15 ethnically diverse populations was consistent with other studies of human divergence and with the archaeological record (Ranjan Deka). Y-chromosome-specific polymorphisms, useful both for paternity testing as well as for evolutionary studies of male lineages, have been studied in Indian (Pandya) and German (Roewer) populations. Conversely, mitochondrial DNA polymorphisms (in the noncoding D loop region) have been analysed to trace maternal lineages (Ovtchinnikova). It may be interesting to compare the mutation rates in mitochondrial DNA with those of the W chromosome, say in chickens, since both are maternally inherited. Alu sequence polymorphisms among Amerindian populations ranging from Alaska to Argentina were used to trace the early human colonization of the Americas (Herrera). Daniel Corach provided a dramatic account of DNA fingerprinting analysis following a mass disaster – the bombing of the Argentine Mutual Israelian Association building in Buenos Aires in July 1994, in which remains of more than 100 victims were analysed within four months. Garofano alerted the forensic community about the possible mistyping of HLADQα during PCR amplification from human remains.

With the recognition that amplification of polymorphic trinucleotide repeat sequences underlies genetic disorders like Huntington disease and fragile X syndrome (Hummerich), studies of minisatellite dynamics have been brought to the forefront of medical research. Progressive amplification of trinucleotide repeats can now explain the previously baffling phenomenon of anticipation, the tendency of these diseases to appear at earlier onset ages and with increasing severity in suc-

cessive generations of a pedigree. Brahmachari cloned 12–84 copies of the CTG trinucleotide (which is amplified in myotonic dystrophy patients) into the *lacZ* gene on a plasmid and found that as the repeat number increased from 36 to 48 there was an abrupt drop in lacZ activity. Plasmids with repeat lengths greater than 36 also showed anomalous electrophoretic mobility on agarose gels which suggested that the repeats compacted the plasmid structure. He proposed that CTG repeats formed a quadruplex structure (as in telomeres) which might block transcription by RNA polymerase.

Jeffreys (anointed the 'pope' of DNA fingerprinting at this meeting) explored why some minisatellite sequences are *not* polymorphic. His analysis of minisatellite variant repeats at the MS32 minisatellite locus showed greater *de novo* mutation rates in sperm samples of Caucasian and Japanese males than in those from African males. The basis for this difference was traced to the 01 site 48 bp upstream of the minisatellite sequence. The presence of the nucleotide G at this site was associated with hypermutation whereas a C depressed the mutation rates. Thus, minisatellite instability was demonstrated to be regulated by a cis element outside the array. The 01 site may act as an initiator of mutation/gene conversion events because hypermutation events showed polarity with respect to 01G. There may be a bias in gene conversion events leading to the replacement of 01G by 01C (a meiotic drive). Such bias may be needed to prevent 01G-induced hypermutation from taking over the chromosome. Lalji Singh reported the Bkm-binding protein's (BBP) specific binding to the Bkm-minisatellite DNA. BBP is but one example of several recently identified minisatellite-binding proteins. It is expressed in a sex- and tissue-specific manner (germ cells of heterogametic sex) since its target sequence (Bkm) is found predominantly on the sex chromosomes (Y and W chromosomes). He proposed that GATA repeats of Bkm bring about a coordinated decondensation of the W and Y sex chromosomes in the germ cells of the heterogametic sex in response to BBP, which may serve as a switch for the activation of the genes present on the W and Y chromosomes. He suggested that different minisatellites may have different functions. It would, of course, be a mistake to assign a generalized function to all minisatellites.

Other discussed examples of medically relevant applications of DNA fingerprinting included diagnosis of infectious diseases such as tuberculosis and malaria (Pearson, Bayoumi). It can be used to distinguish between tumour and normal tissues. Genomic rearrangements (e g. loss of heterozygosity and gene amplifications) can be detected by their effect on the DNA profile (Peason, Garcia-Orad). Selective underreplication of chromosomes in the dividing hepatocytes of regenerating rat liver were shown to produce DNA fingerprint differences from normal cell patterns (Prima). Epplen described his group's efforts to identify DNA polymorphisms in immunorelevant genes as markers for susceptibility to autoimmune disease, particularly rheumatoid arthritis and multiple sclerois.

The potential of DNA fingerprinting has been recognized in sericulture (Nagaraju), pisciculture (Kshitish Majumdar) and, of course, agriculture. DNA polymorphisms have been used as markers for various cultivars of bananas (Bhat), to tag a gene for Ascochyta resistance in chick-pea (Huttel, Sharma), and also in brassicas (Lakshmikumaran), peanut (Prakash), amaranths (Kumar) and Himalayan poppies (Sulaiman). Wayne-Powell described its use for germplasm conservation in coffee and soya beans. DNA fingerprints were also used to identify pathogens and pests of rice such as *Xanthomonas oryzae* (Chowdari) and the gall midge (Ehtesham) and the fungal pathogen of chick-pea *Ascochyta rabiei* (N. P. Singh).

Programs and studies in animal husbandry, wild-life conservation and animal behaviour have also found applications of DNA fingerprinting technologies. It has been used to monitor inbreeding in captive populations of Rhesus monkeys (Ely) and in the endangered Waldraap ibis (Jeffreys). Georges described the use of a bovine genetic map to identify quantitative trait loci (QTLs) that influence milk yield and composition; one QTL was closely linked to the weaver mutation that causes neural degeneration. He also described the callipyge mutation in sheep as an example of a balanced polymorphism. Callipyge sheep have a 'rounded bottom' phenotype, but this phenotype is seen only if the mutation is heterozygous. Homozygous callipyge individuals are indistinguishable from the wild type. The only other instances of the heterozygote being different from the two classes of homozygotes that I am aware of is in transvection in Drosophila. For transvection the paternal and maternal homologues of a gene need to be 'paired' i.e. located at similar chromosomal positions. The mutant phenotype is a consequence of disruption of pairing following chromosome rearrangement and, therefore, is seen only in the heterozygous individual. If callipyge is indeed associated with chromosome rearrangement, it may represent the first example of transvection in mammals. Curiously, callipyge is expressed only when the mutation is paternally derived.

Maynotti-Raymond from Stephen O'Brian's laboratory described the use of DNA polymorphisms to estimate genetic relatedness and phylogenies of the big cats (cheetahs, lions and leopards). The alarmingly low genetic variability found in the Gir lion is due to an evolutionary bottleneck in the early years of this century when its numbers were down to about 20. In cheetahs a bottleneck was estimated to have occurred between 6000 and 13,000 years ago. Miththapala used DNA markers to draw a phylogeny of leopards and showed that island populations (from Sri Lanka and Java) are more inbred than continental populations. DNA fingerprinting was used by Wickings to relate the duration of a dominant male's reign in a brood with the time taken for his daughters to attain sexual maturity. In a poster, she also described the tracking of Western lowland gorillas by DNA fingerprints of hairs collected at nesting sites. The hybridization of the Ethiopian wolf with ferral domestic dogs was monitored by Bruford. He also used DNA from faecal samples to work out the genetic relatedness among baboons in the Amboseli National Park. Tokarskaya used DNA polymorphisms to define subpopulations of Siberian cranes. Kayser examined the human minisatellite markers D12S66 and D12S67 to trace primate evolution. McPartlan described the wide applications of STR markers by the Victorian Institute of Animal Sciences (Australia) for parentage analysis of harness horses, greyhounds and alpacas. Her colleague Even used DNA profiles to study the evolution of horses and pony breeds. Also from Australia, Temple-Smith literally went out on a limb (of a eucalypt) to obtain samples for DNA profiles of koalas.

It was perhaps appropriate that the meeting was formally inaugurated by the Governor of Andhra Pradesh only after the talk by Carleton Gadjusek on an *infectious agent (infectious amyloid)* that leaves no DNA fingerprint. Infectious amyloid proteins follow Koch's postulates and are the causative agents for various encephalopathies and amyloidoses of the brain, including Creutzfeldt-Jakob dementia and bovine spongiform encephalitis. The infectious-disease-causing proteins are derived by conformational changes in normal host precursor molecules that are induced via nucleation with an infective molecule. Thus, there are no differences in the amino acid sequence of the normal and infective variants and hence no underlying genetic differences. Mutations in the precursor protein, however, can increase the likelihood of spontaneous generation of amyloids. Amyloids have also been implicated in cases of hereditary blindness, and can affect heart, gut and *kidneys.*

The conference bore the unmistakable signature of its chairman, Pushpa Bhargava. Two popular talks were open to the general public. One, by Bhargava, illustrated the contributions of Lalji Singh and colleagues at CCMB in fostering DNA fingerprinting awareness even in remote tribal hamlets. The other, by Susumo Ohno, dealt with the persistence of genes even after they have outlived their usefulness (e.g. chicken genes for dentine and tooth enamel). The long half-life of redundant genes (45 million years) explains why ontogeny recapitulates phylogeny as, for example, in the sequential development of three kidneys in human embryonic development.

Fine science blended with fine food (including authentic Hyderabadi fare) and fine art. This included folk dancers of Gujarat (Dr Parul Shah's troupe), an evening at Golconda, followed by qawaalis and a 'chowki dinner' at the nearby Qutb Shahi tombs (courtesy AP Department of Tourism), and a visit to an artists' camp at the Sanghinagar township (built around a polyester plant). The Fourth International Conference on DNA Fingerprinting in Melbourne (December 1996) will surely find TICDF a hard act to follow!

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