DNA technology and its applications in herpetological research and forensic investigations involving reptiles and amphibians

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Abstract. DNA-based technologies, in particular those involved with the identification and screening of DNA polymorphisms, have become a major analytical tool for forensic investigators. These technologies were originally designed for crime scene evaluation and analysis and, more specifically, to the identification of individuals linked to crime scenes. However, the same technologies have subsequently been used to identify polymorphisms capable of discrimination at the individual or species level in a wide range of vertebrates. These polymorphic markers are relevant to a range of research and investigative applications in reptiles and amphibians including population and conservation studies, phylogenetics and forensic analysis. Initially cost-prohibitive, DNA technology is now within the budget of many non-specialised laboratories and field centres. The advent of PCR-based methodologies has allowed the purification and subsequent profiling of DNA from an impressive array of biological materials, including limited amounts of partially degraded field or forensic samples. In this respect, non-invasive sampling of endangered species in the field is of particular interest. Polymorphisms occurring within regions of the mitochondrial genomes of vertebrates are currently being screened for species-specific identification purposes. Databases are under construction that will allow rapid comparison of matching regions of the genomes of many thousands of animal species — with obvious applications in forensic investigations.

Key words: DNA databases; forensic samples; mini and microsatellites; mitochondrial DNA; non-invasive sampling; nuclear and extra-nuclear genomes; single nucleotide polymorphisms; tandemly repetitive DNA.

Introduction

Recent advances in recombinant DNA (deoxyribonucleic acid) technologies, coupled with the availability of relatively cheap equipment which has largely negated the need for highly specialised laboratories, has allowed researchers from many different fields to turn to DNA analysis and in particular, genetic polymorphisms as a tool for the study of biological systems. These studies have included investigations into the evolutionary relationships of individuals and populations both within and
across species and in this respect, classical morphological methods are now in the decline as an increased number of researchers have adopted DNA analysis as the principal tool in phylogenetic research. More recently, the trade in endangered animals and their products (see Shepherd and Abdullah, this series), along with other issues directly related to wildlife crime have increased the need for the development of species-specific markers. DNA markers have proved to be highly valuable in this respect as they are not reliant on morphological detail and are thus capable of identification of distinct species and/or individuals when only limited amounts of tissue are available.

**Nuclear and extra-nuclear DNA**

DNA is found in the majority of cell types in all species and essentially can be divided into two distinct forms — nuclear DNA and extra-nuclear DNA.

In eukaryotes the vast majority of the genome exists in the form of linear chromosomes contained in the nucleus of nucleate cells. The nuclear genome is inherited sexually from both parents and is subject to allelic rearrangement in the offspring due to recombination occurring during meiosis. Nuclear DNA (nDNA) is composed of ‘coding’ and ‘non-coding’ regions. The coding regions are the genes containing the genetic information necessary for the cell to make proteins and RNAs (ribonucleic acids). In general, the coding regions of genes account for only a small percentage of the nuclear genome, with the remainder consisting of non-coding DNA, which as yet, appears to have no direct involvement in protein or RNA synthesis. DNA for which no biological function has yet been ascertained is often referred to as ‘junk DNA’: however, polymorphic regions identified within the non-coding DNA are the most widely used genetic markers and have proved to be invaluable tools for forensic analysis.

Polymorphic regions include hypervariable, repetitive, loci consisting of tandemly repeated core sequence motifs. These repeat regions are hypervariable due to a high mutation rate compared with coding regions. Mutation occurring at the hypervariable loci results in either decreases or increases in the number of repeats of the core motif and appears to be a result of slippage during DNA synthesis (Schlotterer and Tautz, 1992). Whilst the core motif for each loci remains essentially unchanged, the number of repeats may differ at any particular loci, resulting in multiple alleles. For any repeat marker an individual will inherit one allele from each of their parents and this variation in the number of tandem repeats (VNTR) gives rise to a high degree of heterozygosity within a population at VNTR loci.

Tandemly repetitive loci are further categorised on the basis of the size of the repeating unit. Short tandem repeats (STRs) consisting of simple repeat units of approximately 2-9 bases are termed microsatellites, with larger repeat units termed minisatellites. A significant number of microsatellite loci have been identified for a range of species which demonstrate a high degree of variation between individuals. The discriminating nature of STR loci forms the basis of modern forensic DNA-profiling in humans (Edwards et al., 1991) and the highly polymorphic nature of