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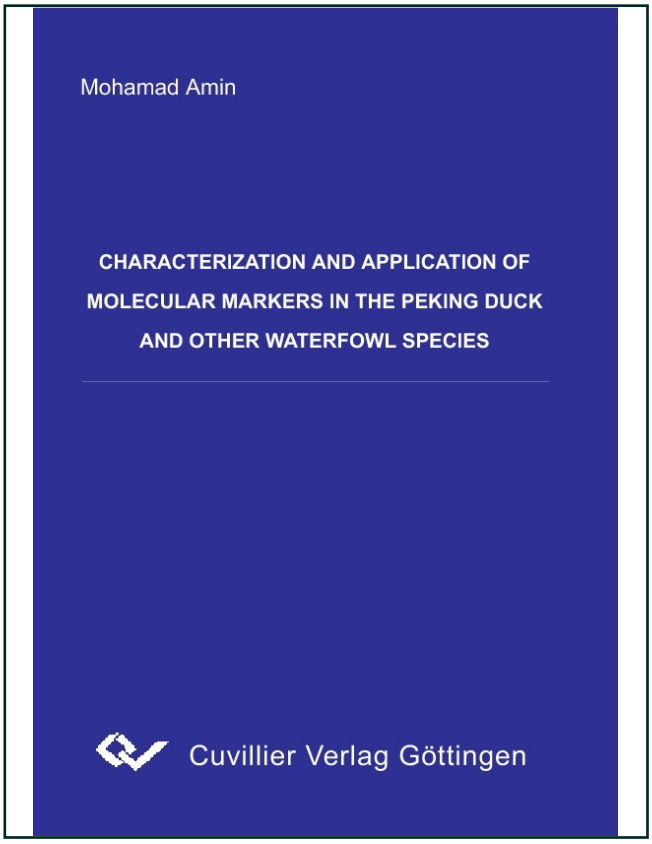
Characterization and application of molecular markers in the Peking duck and other waterfowl species



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1. Introduction

In the four decades since the discovery of DNA, molecular genetics and biotechnology have undergone a revolution in the research and application of genetic testing techniques. Researchers can use DNA markers to follow individual traits in different environments and hosts, increasing our understanding of the constitution, diversity, and evolution of genetic material (Caetano-Anollés & Gresshoff 1997).

The advent of DNA marker technology made it possible to detect naturally occurring polymorphisms at the DNA level. It became a revolutionizing tool for both applied and basic diagnostic studies of plant, animal and human genomes as well as for microorganisms. The various established and widely used DNA marker techniques include approaches detecting restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), single-nucleotide polymorphisms (SNPs) also mini- and microsatellite DNAs (variable number tandem repeats, VNTRs; simple sequence repeats, SSRs). Other, more specific approaches exploit mitochondrial, chloroplast or ribosomal DNAs.

1.1 DNA markers

Even in those organisms in which the maps appeared to be “full” of loci of known phenotypic effect, measurements showed that the recombinational intervals between known genes had to contain vast amounts of DNA. These intervals, or gaps, could not be mapped by linkage analysis, because there were no markers in those regions. Large numbers of additional genetic markers were needed, which could be used to fill in the gaps to provide a higher-resolution map. This need was met by the discovery of various kinds of molecular markers. A molecular marker is a site of heterozygosity for some type of neutral DNA variation (Griffith *et al.* 1999). Neutral variation is that which is not associated with any measurable phenotypic variation. Genetic markers are defined variable DNA or protein sequence which can be used in genetic mapping and analysis. The optimal characteristic for these markers are easily to assay from small amount of material, highly variable and of codominant inheritance.

In the past two decades, molecular marker techniques have been developed as a direct result of the needs of molecular genetic analysis. These techniques range from molecular assays for genetic mapping, gene cloning and marker assisted animal breeding to genome fingerprinting and for the investigation of genetic relatedness. Genetic markers are based on DNA polymorphisms in the nucleotide sequences of genomic regions are either defined by restriction enzymes, or two priming sites.

Mitochondrial DNA

The discovery of restriction endonucleases and the advent of recombinant DNA technology have greatly prompted studies on mtDNA from a variety species. The very simple procedure to isolate, small genome size, abundance in eukaryotes and the unisexual mode of inheritance have predestinated it as an object in evolutionary, population genetic and molecular studies (Geldermann & Ellendorf 1990). The two features of mtDNA make it particularly valuable for molecular genetic studies. First, evolution of mtDNA occurs primarily as single base pair substitutions, with only infrequently major sequence rearrangements (Wolstenholme 1992). Second, the rate of mtDNA evolution appears to be as much as 10 times fasters than that of nuclear DNA (Brown *et al.* 1979). These features facilitate the use of mtDNA as a tool for genetic marker (Avise *et al.* 1979; Brown *et al.* 1979).

Advantages of using mt-DNA sequence include:

- The DNA molecule in most animals is relatively small and easy to isolate.
- It is present in multiple copies per cell; therefore, older and less well preserved samples are still likely to yield useful information.
- The mitochondrial genome does not undergo recombination, so it is more likely to show a clean branching structure to its gene trees.
- It evolves rapidly.

But some primary problems with mtDNA are:

- The absence of recombination means that the gene tree constructed from any mitochondrial DNA gene will reflect just a single realization of the genealogical process. As such, the data will not be as informative about species or population trees, as, e.g. a dozen nuclear genes.

- Much of the work with mtDNA has been on the control region sequence. While this region is highly variable, the variability occurs at a subset of sites that are so mutable that multiple substitutions often occurs (Hartl & Clark 1997).

The mitochondrial (mt) cytochrome-*b* gene has been used to study the evolution and phylogenic relationships of many animals, such as birds, mammals and fish (Aquadro & Greenberg 1983; Chikuni *et al.* 1995; Edwards & Wilson 1990; Irwin *et al.* 1991; Kocher *et al.* 1989). The major reasons of mtDNA cytochrome-*b* match for phylogenetic marker are: it is slow in term of amino acid substitutions; the rate of evolution for silent substitution in third codon position is similar to that of other mitochondrial gene (Meyer 1994).

Restriction fragment length polymorphisms (RFLPs)

The word 'fragment length polymorphism' means DNA pieces of different length. Cleavage of DNA from different individuals with restriction enzymes produced differing sets of restriction fragments. This polymorphism type described previously has to be put down to the various lengths of restriction fragments apparently. This technique uses cDNA or other cloned single-copy DNA elements as radioactively labeled probes in hybridization with restricted genomic DNA. The combination of DNA probe and genotype-specific restriction enzyme pattern reveal a RFLP. RFLPs are co-dominant and identify a unique locus and therefore, are very informative. When cDNA with known gene function are used as markers, the chromosomal position of the specific gene or genes can be identified (Page & Holmes 1998; Nicholl 1995).

Randomly amplified polymorphic DNA (RAPDs)

RAPD (randomly amplified polymorphic DNA) is a set of several genomic fragments amplified by a single PCR primer set. It is somewhat variable from individual to individual; heterozygotes for individual fragments can act as markers in genome mapping (Griffith *et al.* 1999). A single PCR primer designed at random will often used by changing amplify several different regions of the genome. The single sequence "finds" DNA bracketed by two inverted copies of the primer sequences. RAPD polymorphisms result from DNA sequences variation at primer binding site and from DNA length differences

between primer binding sites. That technique has been widely used for screening of DNA sequences-based polymorphisms at very large number of loci, because it requires small amounts of DNA (15-25 ng), is a radioactivity free assay and can be performed in several hours. But the unpredictable behavior of short primers which is affected by numerous reaction conditions, the inheritance in a dominant manner and population specificity are the main disadvantages of RAPDs.

Amplified fragment length polymorphisms (AFLPs)

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters, (ii) selective amplification of sets of restriction fragments, and (iii) gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide gels. The AFLP technique provides a novel and very powerful DNA-fingerprinting technique for DNAs of any origin or complexity (Vos *et al.* 1995; Blears *et al.* 1998; Reineke *et al.* 1998).

The technique for fingerprinting may be used as a tool to identify a specific DNA sample or to asses the relatedness between samples. Fingerprinting is also used as the source for genetic markers to generate linkage maps or to identify molecular markers linked to phenotypic traits and or genetic loci. AFLPs are predominantly not co-dominant and still expensive to generate because the fragments are detected by silver staining, fluorescent dye or radioactive (Areschenkova 2000).

Single-nucleotide polymorphisms (SNPs)

SNPs comprise the largest set of sequence variants in organisms. This variant can be expressed either as a deletion, an insertion or a substitution. It could be identified by various methods, e.g. comparison of sequence and heteroduplex hybridization. Kwok & Gu (1999) proposed many reasons why SNPs have become so important within last years. First reason, SNPs happen very frequently within genome. This fact will increase the ease with which SNPs are studied. Seconds, the mutation rate from generation to generation is very low. Positional cloning based on SNPs may accelerate the identification of human disease traits and a range of biologically informative mutations. The recent application of high-density oligonucleotide arrays to allele identification has made it feasible to genotype thousands of biallelic SNPs in a single experiment. It has yet to be established, however, whether SNP detection using oligonucleotide arrays can be used to accelerate the mapping of traits in diploid genomes (Cho *et al*. 1999; Albert *et al.* 2002).

Microsatellites

Few types of DNA sequences have gained interest among which a wide range of scientists as a class of simple tandem repetitive DNA commonly referred to as microsatellites (Brohede & Ellegren 1999). Microsatellite, or simple sequences length polymorphisms (SSLPs), short tandem repeats (STRs), simple sequence motifs (SSMs), sequence target microsatellites (STMs), is a class of repetitive sequences which are widely-distributed in the genomes of apparently all eukaryotes. They are genetic markers that can be useful in addressing questions at a variety of scales, ranging from extremely fine grained to the fairly coarse grained. More specifically, these genetic tools can help solving problems ranging from individual-specific such as kinship (Queller *et al.* 1993), determining gender, to question of relatedness and parentage, genetic mapping, the genetic structure of population and up to comparisons among species (McDonald & Potts 1997; Dib *et al.* 1996). Traditional genetic surveys of microsatellite loci capitalize upon SSR variation because it is easy to score DNA fragment by size. Length variation is conspicuous and usually the sole criterion employed to characterize allelic diversity at loci displaying variable number of tandem repeat (Orti *et al*. 1997).