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CHICKEN DNA FINGERPRINTING WITH M-AFLP AND S-SAP MARKERS DESIGNED ON SATELLITE GENOMIC REGIONS

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The present research deals with the development of an innovative system of forensic genomics for the chicken DNA fingerprinting based on the analysis of repetitive sequence families (both microsatellites and minisatellites) with the aim of identifying and cloning breed-specific molecular markers. The relatively small genome of chicken (1.200 Mpb) contains around 15% of repetitive DNA organized as short tandem repeats as well as numerous families of interspersed repeats, mainly derived from transposable elements and located over all chromosomes (autosomes, macro-, intermediate, and micro-chromosomes, and sex chromosomes) even though not uniformly. It has also been proved that micro-chromosomes contain more single-copy sequences and less repeated sequences than macro-chromosomes, and that sex chromosomes are very rich of highly repetitive DNA. On the basis of this information, DNA sequences of the repetitive elements were considered very useful to set up analysis systems of forensic genetics for a reliable identification of species, breeds and also single individuals on the basis of the banding patterns generated. The detection of these elements in the chicken genome was based on the S-SAP and M-AFLP systems, derived from the more widely known AFLP technology. The approach provided for the amplification of genomic cleaved fragments, ligated to specific adaptors and pre-amplified with selective primers, using an AFLP primer in combination with a primer that anneal to a repeated element (i.e., PO41) or with a primer anchored to a microsatellite motif (e.g., $(AGC)_n$ and $(CA)_n$). Genomic DNA fingerprints were generated in a total of 84 individuals belonging to six local breeds (Ermellinata di Rovigo, Pépoi, Robusta Lionata, Padovana, Polverara, Robusta Maculata) and one commercial broiler (Golden). Highly discriminant fingerprints based on microsatellite and minisatellite DNA regions were scored using the primer combinations $CAG(CA)_8$/EcoRI+A and PO41/EcoRI+A, respectively. The effective number of alleles per locus was equal to ne=1.570. Total Nei's genetic diversity over all genomic loci was Ht=0.334, whereas the mean genetic diversity of single breeds was Hs=0.162. The genetic variation was shown to be equally distributed within and among breeds (G_{st}=0.515), suggesting that the local breeds conserved well-separated their gene pools over time. The construction of UPGMA dendrograms and the definition of centroids according to the principal component analysis were also performed using total and mean Dice's genetic similarity matrices. The mean genetic similarity coefficients within and between local breeds were 0.799 and 0.557, respectively. A number of markers highly polymorphic among breeds as well as a few breed-specific markers were sequenced and used as queries for public database interrogations. Chicken genome retrievals revealed significant similarities with genic and intergenic sequences of known chromosome position and primary structure homologies with known gene products. It is worth mentioning a 227 bp-long clone that scored a highly significant similarity with a gene located on chromosome 10 coding for a myosin-domain containing protein. In conclusion, the setting up of a molecular reference system seems to be feasible and

suitable both for the precise identification of the single breeds and for the evaluation of the genetic relationships among the different breeds and the commercial broiler.