Evolutionecology

Summary The article discusses the selection of proper strategy to deal with evolution and genetic variation among closely related species. It was clearly demonstrated that use of right technique like PCR-RFLP makes analysis more rapid and gives good correlation between classical RFLP data and sequence based phylogenetic distance analysis. It also validates use of mitochondrion DNA as genetic marker for parental identification and for identification of hybridization. In the conclusion, the author demonstrates how different methods of phylogenitic analysis give different results Thus, it is very important to draw right conclusion considering limitations of each techniques.

Paper starts with the hypothesis demonstrating mitochondrial DNA as primary tool for investigation of evolutionary diversion among closely related species and development of new approach in terms of PCR-RFLP based rapid and inexpensive techniques to established phylogenitic correlation among different species. They also investigated correlation between two different approaches and indicated that data obtained by two different techniques may not be identical and hence caution must be taken to interpret them. For validation of hypothesis the authors selected four avian sp. found in North America, those having high rate of hybridization namely, Dendroica occidentalis, D. townsendi, D. virens, and D. nigrescens. To investigate above mention hypothesis the first experiment was based on classical RFLP based technique. The total mitochondrial DNA were Isolated and digested with 14 restriction enzymes to obtained band pattern which was subsequently analyzed by David L. Swofford's paup* 4. 0d64 program for calculation of Nei-Li distances. Similarly, for sequences based analysis, three genes located on two sites on mtDNA were selected and sequenced, namely

681bp cytochrome oxidase I, and 1074bp ATP synthase8 and 6 genes from 30 representative warbler individuals. All these sequences were used to calculate K2 and MP analysis. For development of PCR-RFLP based techniques mt DNA of two species were scanned in regions where COI and ATPase were located for identification of site having nucleotide sequence variations. Amongst the various sites few restriction sites were selected, those which are recognized by commercially available restriction enzymes. Out of six diagnostic sites, on COI, two sites were selected having restriction site for XmnI. MspI and AluI. Based on sequence information, primers were designed to amplify those regions and after amplification PCR product was used for RFLP analysis using 4 restriction enzymes.

Comparison of sequence data with RFLP data shows good correlation when autocorrelation function was applied but once autocorrelation function was removed some differences among species origin were observed. Chronological clock gave different time line for origin of species in case of RFLP and sequence based data. For evaluating use of PCR-RFLP system they employed two populations of townsendi and occidentalis. Ancestral townsendi were represented by 39 individuals from eastern British Columbia, north central Alaska, and eastern Oregon. Ancestral occidentalis were represented by 19 individuals from southern Oregon and northwestern California. Two populations from the phenotypic centers of the current hybrid zones were sampled, including 15 individuals from Skamania County, Washington, and 18 individuals from Klickitat County, Washington. The PCR-RFLP and subsequent band profiles validates use and performance of this new techniques.