

# Additional Molecular Evidence that The Royal Palm is Probably a Turkey Breed

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In earlier investigations, we reported the uniqueness of Royal Palm from other commonly raise heritage turkeys. Here, we provide evidence from screening using primers specific for the Melanocortin 1 (MC1R) Receptor gene (located on turkey chromosome 13, accession number NC\_015023). Five heritage strains, including Broad Breasted Bronze (48), Blue Slate (40), Midget White (40), Royal Palm (45), and Spanish Black (48) were included in the analyses of approximately 3300 base pairs of the *MC1R* gene. Seven SNPs were identified and validated. Among the haplotypes identified, only Royal Palm had unique haplotypes. When combined with previous investigations, we believe that the Royal Palm may be a breed and not a strain. It may therefore be the most viable resource for introgression with a goal of genetic improvement.

Keywords: Turkey, Varieties, Molecular Phylogeny.

- For the last 15 years, we participated in a project funded by the American Livestock Breed Conservancy that sought to define genetic and phenotypic relatedness among heritage turkeys. In the present work, data was pooled from analysis involving diverse DNA markers to determine:
  - The relatedness among heritage strains and
  - The relatedness can offer clues of whether these populations were breeds or strains.
  - resistance. In the present work, MC1R-based haplogroups were used to further distinguish among heritage turkeys.

- Animals:** 25-50 birds were used from each of seven varieties including Bourbon Red, Royal Palm, Narragansett, Blue Slate, Beltsville small white, and Spanish Black. Birds from a commercial stock as well as two wild varieties were also used in earlier molecular analyses. Birds from the heritage varieties were obtained from commercial hatcheries as day-old birds and raised at the Virginia Tech Turkey Farm using standard protocols.
- Molecular markers:** The DNA marker systems used were MC1R-based SNPs and in earlier studies RAPD, microsatellites, SNPs within ESTs, and mitochondria DNA nucleotide variants within the D-loop and 16s rRNA.
- Phylogenetic analysis:** Default models built into Clustal-W and PAUP\*4.0 computer programs were used. Both programs have as a default a one-parameter model known as the "Jukes-Cantor."

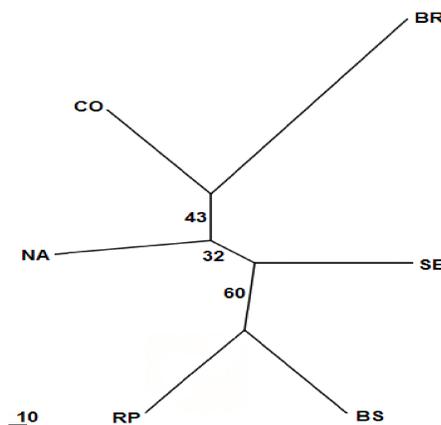
The markers used here have previously been used to evaluate relatedness among chickens obtained from the same breeder (Privett Hatcheries). On average, 5 alleles per microsatellite locus were detected when multiple breeds were compared. Similarly, SNPs within the mtDNA were at a much higher frequency than in the turkey strains compared here. In earlier work, we inferred from the low level of diversity among the strains across many different loci including the mitochondria, that the turkey varieties are too closely related to be considered breeds in the classic sense and when compared to chickens. The MC1R-based haplogroups data (figure on the left), however, suggest otherwise.

## Reference:

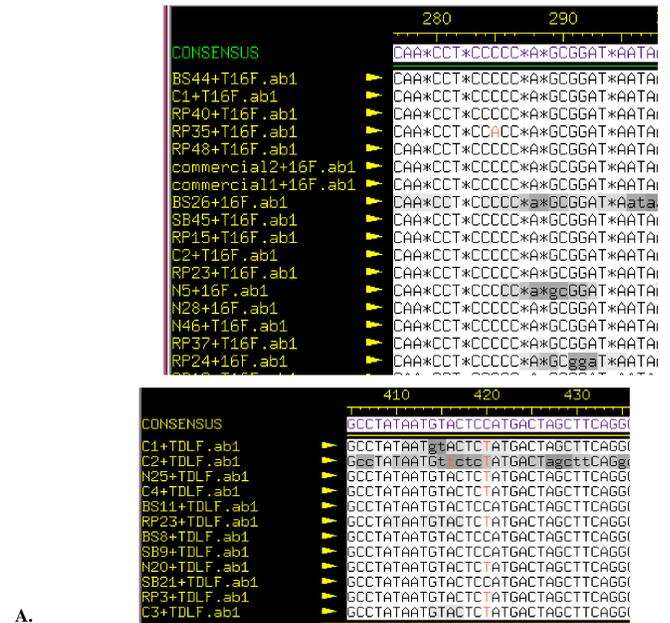
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Heritage turkeys have become of interest to the consumers in the USA and other developed countries because of the perception that they care, unlike commercial/industrial birds, "more organic" (Parrish, 2002; and Shriver, 2003). As their number increases, and more farmers beyond fanciers raise them for the broader market, the need to increase biological information about them increases. Although the turkey varieties are considered a single breed (American Poultry Association, 2001), evidence is emerging about significant strain differences among the populations (Hartman et al., 2006) and between the heritage turkeys and commercial birds (Gyenai, 2005). In his unpublished thesis work, Gyenai (2005) evaluated phenotypic differences in commercial and Heritage turkey populations for their response to toxic levels of furazolidone in order to make inferences about the genetic basis of the incidence and severity of dilated cardiomyopathy (DCM) in turkeys. Variety differences in the turkey's response to diets containing furazolidone were observed, suggesting that an animal's response to furazolidone-induced DCM is genetically based. More recently, among Heritage varieties, Hartman et al. (in press) observed significant strain differences for plasma uric acid concentration, a biomarker for diverse phenotypes including oxidative stress. Genetic variation within and among commercial turkey populations was also previously evaluated by others including Ye et al. (1998). Although Smith et al. (2005) also analyzed the genetic relatedness of 5 heritage turkey varieties, the relatedness between the commercial and heritage turkeys has never been investigated. Information regarding the genetic relatedness between commercial and heritage turkey varieties can be used for genetic improvement of the different turkey strains such as the introgression of novel genes important for economic traits including disease

**Fig 1:** A neighbor joining tree using Nei's distance for all population sample + 10 microsatellite loci. Bootstrap values indicated at the nodes were calculated from 100 re-samplings. Where NA, BR, BS, SB, RP, and CO represent Narragansett, Bourbon Red, Blue State, Spanish Black, Royal Palm and commercial strain, respectively.



**Fig 2:** Consed-aligned views showing (A), a C-A SNP in the ND6 gene and (B), a C-T variant in the D-loop of the Turkey mitochondria. In 2560 bp of the mtDNA, sequence



A.

