GENOMIC VARIABILITY AND MHC CLASS IIβ_1 CHARACTERIZATION IN A REINTRODUCED POPULATION OF EASTERN WILD TURKEYS

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PREFACE

This thesis is composed of two primary chapters, each examining a biological question about a population of eastern wild turkeys in southeastern Oklahoma. Although questions addressed in these chapters are scientifically diverse, they share one important commonality in that they were approached using molecular tools now available within the growing field of conservation genetics.

Following an introductory chapter, Chapter Two discusses levels of inbreeding and genetic subdivision within the study population and is written in manuscript format for the journal to which it has been accepted for publication, *The Southwestern Naturalist*. Chapter Three also is written in manuscript format, with all reference materials separate from those for chapter two. Chapter three will be submitted as a short communication to the journal *Animal Genetics*, and characterizes the Class $II\beta_1$ antigen binding site of the Major Histocompatibility Complex in wild turkeys.

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Chapter 1

INTRODUCTION

Human activities and decisions increasingly influence wildlife populations. This is particularly true for game species exposed to seasonal harvest, reduction and fragmentation of native habitat, and transgeographic reintroductions (Scribner 1993). These perturbations affect population size and sex and age composition and consequently levels and partitioning of genetic variation within populations (Leberg 1990a; Scribner 1993). Severe and multi-generational population decline within a species can be genetically deleterious because it may reduce the overall amount of heritable genetic variation at both the subpopulation and population level, thereby increasing the potential for genetic drift and inbreeding depression (Falconer 1983). Theoretically, reduced genetic variability limits a species' ability to adapt to changing environments making it more susceptible to extinction through random events (Lande 1988). Furthermore, reduced genetic variability increases the likelihood for detrimental or inferior alleles to occur at high frequency or to become fixed within a population, resulting in reduced fitness (O'Brien 1994; Gray 1995).

The eastern wild turkey (*Meleagris gallopavo silvestris*) population was extirpated throughout most of its historical range in Oklahoma in the early part of the 20th century due primarily to overharvest and habitat loss (Masters and Thackston 1985). Following decades of restoration efforts by wildlife management agencies, the estimated population of eastern wild turkeys in southeastern Oklahoma had rebounded to approximately 6,200 by 1980, sufficient to resume public hunting (Thackston 1980). However, since 1980 the population declined to an estimated 1,450 birds in 1994,

representing a more than 76% reduction from previous levels (Dinkines & Smith 1996). In an effort to identify factors affecting survival of the eastern wild turkey in the region, several studies were initiated on the Pushmataha Wildlife Management Area (PWMA) examining aspects of animal fitness.

The findings discussed in this work address two factors potentially influencing fitness of wild turkeys in the region: overall genetic variability and structure within the population and allelic polymorphism within the Major Histocompatibility Complex (MHC) Class IIβ_1 locus, a region critical to immune function. Little is known about the organization and phylogenetic relationship of MHC alleles of birds divergent by evolution or habitat (Jarvi *et al.* 1996). This study provides unique sequence data for MHC Class IIβ_1 alleles from a North American galliform. In concert with detailed sequence information now available for Class II genes in Old World galliforms, (Jarvi *et al.* 1996, Wittzell *et al.* 1994), sequence data provided by this research may help contribute to the understanding of MHC evolution between taxonomically close avian groups.

Chapter 2

GENETIC ATTRIBUTES OF A DECLINING POPULATION OF REINTRODUCED EASTERN WILD TURKEYS (MELEAGRIS GALLOPAVO SILVESTRIS)

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ABSTRACT--The eastern wild turkey (Meleagris gallopavo silvestris) experienced population declines in the United States beginning in the 19th century and continuing through the early 20th century. Transgeographic reintroduction efforts have reestablished eastern turkeys throughout most of their historic range but effects of reintroductions on genetic composition of resulting populations are unclear. Most reintroduced populations are maintaining self-sustainable populations. However, for unknown reasons, the population in southeastern Oklahoma decreased by 77% from 1980 to 1994. We used multi-locus DNA fingerprinting to evaluate levels of similarity, heterozygosity, and allelic diversity within and among wintering flocks occurring on the Pushmataha Wildlife Management Area in southeastern Oklahoma. Results from two restriction enzyme-probe combinations revealed mean similarity, heterozygosity, and allelic diversity similar to those in other outbred avian populations. Therefore, the

decline of wild turkeys in southeastern Oklahoma is not related to reduced genetic variability, but may be due to biotic and abiotic factors not accounted for in this study.

Populations of wildlife are influenced increasingly by human activities that reduce and fragment habitat. This is true particularly for game species exposed to seasonal harvest and transgeographic reintroduction (Scribner, 1993). In the United States, anthropogenic factors have resulted in extirpation of several abundant game species in all or part of their historic range. Notable examples include passenger pigeons (Ectopistes mirgratorius), prairie chickens (Tympanuchus sp.), American black bears (Ursus americanus), and American bison (Bos bison). Similarly, the eastern wild turkey (Meleagris gallopavo silvestris), once abundant and widespread throughout the eastern United States, was extirpated from many regions during the 19th century as a result of human exploitation (Leberg, 1990a). Eastern wild turkeys probably reached their lowest numbers in the late 1930s, existing in only 19% of their former range (Mosby and Handley, 1943; Bent, 1963; Mosby, 1975). However, reintroduction programs were successful in reestablishing turkeys in most parts of their historic range (Leberg et al., 1994).

Although transgeographic reintroductions are an important and commonly used management tool for restoration of game species, several studies disagree on how reintroductions affect genetic composition of resulting populations. Many consider reintroductions to be primarily responsible for the recovery of white-tailed deer (Odocoileus virginianus) in the southeastern United States (Barick, 1951), but Kennedy et al. (1987) and Ellsworth et al. (1994) concluded that reintroductions did not significantly alter the genetic composition of extant populations. In contrast, based on their study of genetic structure of populations of white-tailed deer and wild turkey in the southeastern United States, Leberg et al. (1994) concluded that reintroduced individuals had significantly altered the genetic composition of resident populations. They suggested

that differences between their findings and those of Kennedy et al. (1987) and Ellsworth et al. (1994) may be related to different reintroduction histories among populations examined or statistical analyses used to determine how geographic proximity and reintroduction influence genetic structure of populations. Because reintroduction most likely will continue to be an important management tool for recovery of many declining species (Griffith et al., 1989; Leberg et al., 1994), further study of how reintroductions affect genetic composition of resulting populations is needed to evaluate the effectiveness of this management practice.

Eastern wild turkeys essentially were eliminated from southeastern Oklahoma for many decades but, through protection efforts and reintroduction of turkeys from Arkansas and Missouri, the population in southeastern Oklahoma was estimated to number approximately 6,200 in 1980 (Thackston, 1980). Subsequent to 1980, the regional population declined to an estimated 1,450 birds by 1994, a 77% reduction from previous levels (Dinkines and Smith, 1995); the cause of this decline is unknown. Using eastern wild turkeys in southeastern Oklahoma as a model for many reintroduced populations throughout the United States, our purpose was to address the possibility that low intrapopulation genetic variation may be one factor contributing to the decline of eastern wild turkeys in this region. Reduced genetic variability increases the likelihood for deleterious alleles to achieve high frequency or become fixed within populations, potentially resulting in reduced fitness (O'Brien, 1994; Gray, 1995). Reduced genetic variability also is thought to limit a population's ability to adapt to changing environments, making it more susceptible to extinction through random events (Lande, 1988). We examined levels of genetic variability in this population to determine if loss of genetic diversity was contributing to the decline of turkeys in southeastern Oklahoma. To accomplish this, we analyzed patterns of genetic variation based on multi-locus DNA fingerprinting (Jeffreys et al., 1985) within and among eight winter flocks of turkeys.

MATERIALS AND METHODS--Approximately 30 trap sites across the Pushmataha Wildlife Management Area (PWMA), in Pushmataha Co., Oklahoma, were baited daily with cracked corn from December through April 1995-1996 to attract flocks of wintering turkeys. After consistent feeding patterns were established, preset cannon or rocket nets (Hawkins et al., 1968) were fired from a nearby blind over flocks primarily composed of females. Upon capture, turkeys were placed in protective boxes (National Wild Turkey Federation) to reduce stress. Heparinized syringes were used to collect blood samples from brachial veins, and three drops of blood from each individual were placed in 5 ml of lysis buffer for DNA isolation (Longmire et al., 1997).

Genomic DNA (10 μg) from 48 females and three males was digested separately with an excess of restriction endonucleases Hinf I and Hae III in recommended buffers overnight at 37°C. Following heat deactivation of restriction enzymes, approximately 30 ng of a 1-Kb size standard were added to the digested DNA for each sample. Restricted genomic DNA, along with the internal size standard, was electrophoresed in 0.8% agarose TBE gels until the 4-Kb marker approached the end of the gel. For visual reference, the first and last lanes of each gel contained 0.5 μg of 1-Kb and 0.1 μg of λ DNA/Hind III standard size markers. DNA fragments were transferred to nylon membranes with 20X SSC (modified from Southern, 1975) and fixed by baking at 80°C for 2 h. For estimates of within-flock genetic variation, all samples within a flock were adjacent on the same gel, eliminating among-gel error. For estimates of among-flock genetic variation, at least one member of each flock occupied an adjacent lane with at least one member of all other flocks. This was achieved by randomly loading (without replacement) two to four representatives of each flock per gel for a total of four gels.

Membranes adhering Hinf I fragments were probed with the simple microsatellite repeat oligonucleotide $(GT)_n$, and membranes adhering Hae III fragments were probed with the minisatellite probe pV47-2 (Longmire et al., 1990) using an ECL non-radioactive detection kit (Amersham). Fragments were visualized via autoradiography.

Subsequently, membranes were stripped and hybridized with 1-Kb DNA size standard to develop lane-specific migration curves against which molecular weights of all detectable fragments were estimated. Size for all fragments between 4- and 12-Kb was determined by using a lightbox, transparent metric ruler, and lane-specific standard migration curves. Because of variance in fragment migration across a gel, size categories (bins) were constructed in which bands of approximate size were grouped (Budowle et al., 1991). Bins were calculated based on variance in band migration of distantly spaced replicates of several individuals on the same gel. Approximately two times the standard error for the largest fragments (10 - 12 Kb, which exhibited the most variance) was used as a standard bin size. Because the largest observed bin was 67 bp, we conservatively set the bin size at 100 bp. All bands falling within a bin were considered homologous and unlinked; the resulting presence/absence data were converted to binary format for data analysis.

Fingerprint data were analyzed with the software program Gelstats (Rogstad and Pelikan, 1996). Parameters estimated were mean number of bands per individual, number of loci, number of alleles per locus, allelic frequency, bias-corrected heterozygosity (Jin and Chakraborty, 1993, 1994), and percent similarity (Lynch, 1990). A two-sample t-test was used to test for significant differences between within-flock and among-flock similarity values for each probe. We tested for significant correlation between geographic distance and genetic similarity among flocks using the Mantel analysis option of NTSYS (version 1.5; Rohlf, 1990).

RESULTS--Mean number (± SD) of bands scored per individual was 18.36 ± 1.20 for Hinf I/$(GT)_n$ and 21.11 ± 3.07 for Hae III/pV47-2 (Table 1), with the total number of sizably different bands per gel averaging 68.6 and 70.6, respectively. Mean within-flock similarity was 0.377 ± 0.050 for Hinf I/$(GT)_n$ and 0.439 ± 0.066 for Hae III/pV47-2 (Table 1); mean similarity among flocks was 0.386 ± 0.028 for Hinf I/$(GT)_n$

and 0.455 ± 0.027 for Hae III/pV47-2 (Table 2). Comparison of mean genetic similarity within and among flocks revealed no significant difference for both probes (Hinf I/$(GT)_n$, $t = 0.388$; Hae III/pV47-2, $t = 0.255$; $P > 0.5$ for both probes). Geographic distance between flock locations ranged from 0.71 to 7.39 km, and no significant correlation was detected between genetic and geographic distance among flocks (Hinf I/$(GT)_n$; $t = -0.44$, $P = 0.330$; Hae III/pV47-2: $t = -0.039$, $P = 0.399$).

DISCUSSION--Mean within-flock similarity for wild turkeys on PWMA for microsatellite and minisatellite loci was similar to other outbred avian populations (0.30 - 0.47; Burke and Bruford, 1987; Wetton et al., 1987; Meng et al., 1990; Rave et al., 1993), and comparison of within and among flock similarity values detected no significant differences. These results indicate that wintering female flocks of turkeys on PWMA do not comprise closely related females, and suggest that gene flow (which distributes genetic variation and prevents differentiation) frequently occurs among flocks. This is inconsistent with the conclusion of Boone and Rhodes (1996) that genetic differentiation among flocks of eastern wild turkeys should be most evident in groups of wintering females, along with the implication that "family units" of related females are socially cohesive over long periods of time. Dispersal of two juvenile females 6.2 km from their home range in southeastern Oklahoma (Bidwell, 1985) further supports our interpretation of high levels of gene flow via movement of females between flocks. The population of turkeys at PWMA (and surrounding areas) does not possess high levels of band sharing as would be predicted for a population experiencing severe inbreeding.

Low genetic variation is assumed to threaten the viability of a population (Leberg, 1990a). This assumption is based on the observation that individuals that have lost substantial heterozygosity due to inbreeding generally exhibit depression of fitness traits such as growth, survival, and fecundity (O'Brien et al., 1985; Allendorf and Leary, 1986). Therefore, all other factors being equal, a population of highly heterozygous individuals

would be expected to exhibit greater viability and fecundity than a population of less heterozygous individuals (Quattro and Vrijenhoek, 1989; Leberg, 1990a). Mean heterozygosity for the minisatellite and microsatellite loci examined was 0.72 and 0.64, respectively; higher than that reported for vertebrate populations founded by similar numbers of individuals. For example, populations of Rocky Mountain bighorn sheep (Ovis canadensis) founded from 12 individuals have heterozygosity values of 43% (Forbes et al., 1995) and cheetahs (Acinonyx jubatus), thought to be suffering from reduced fitness due to an ancient bottleneck of as few as two individuals, maintain 39% heterozygosity (Menotti-Raymond and O'Brien, 1995). Eighteen adult koalas (Phascolarctos cinereus) were used to re-establish a population that now exhibits about 33% heterozygosity (Houlden et al., 1996), and a population of northern hairy-nosed wombats (Lasiorhinus kreftii), after passing through a bottleneck of 20-30 individuals, now has 27% population heterozygosity (Taylor et al., 1994). Citing avian examples, heterozygosity within wintering female flocks of PWMA turkeys is higher than reported within four declining coastal populations of clapper rails (Rallus longirostris; 0.247 - 0.421; Fleischer et al., 1995). Observed heterozygosity values for PWMA turkeys are similar to the 72% heterozygosity reported for the population of clapper rails at the Salton Sea (Fleischer et al., 1995) and the 72% to 85% heterozygosity reported for captive populations of the endangered Siberian crane (Grus leucogeranus; Tokarskaya et al., 1995). Therefore, based on DNA fingerprint analysis using two evolutionarily distinct probes and comparison with other DNA fingerprint studies of avian populations, reduced fitness associated with low levels of heterozygosity cannot explain the current decline of eastern wild turkeys in southeastern Oklahoma.

Because the turkey population was thought to be extirpated from southeastern Oklahoma in the early 1900s, and reintroductions often were made with few individuals, the low levels of band-sharing and high heterozygosity in the PWMA population were unexpected, especially considering that previous allozymic studies of 49 populations of

wild turkeys from the eastern United States demonstrated virtually no intrapopulation variation (Leberg, 1991; Leberg et al., 1994). Current levels of genetic variation within the PWMA population probably resulted, in part, from gene flow in the initial stages of population growth between reintroduced founders and other sources (e.g., nearby turkeys from other reintroduction events in Oklahoma and Arkansas or preexisting native turkeys).

Leberg (1990a) demonstrated that the rate of increase of a population following reintroduction can affect heterozygosity within populations. Populations that grow slowly and remain small will lose genetic variability to genetic drift more rapidly than populations that grow quickly, because many more matings in the initial generations are between closely related individuals (Nei et al., 1975; Leberg, 1990b). The PWMA population was reestablished with nine turkeys in the early 1970s, and the population had attained a size sufficient to resume hunting by 1980. This suggests that the population grew rapidly and any loss of genetic variability from founder effect probably was minimal.

Given that the initial rate of growth following reintroduction was rapid and genetic variability remains high, why have turkey populations in southeastern Oklahoma declined so dramatically since 1980 while other populations of reintroduced wild turkeys maintained sustainable numbers? Leberg (1990a) showed that a founding population size of 10 or more individuals should retain greater than 95% of the heterozygosity present in the source population. The nine individuals used as founders for the PWMA population would be expected to retain 94% of the heterozygosity of the source population (Wright, 1969; Leberg, 1990a). Although a reduction in population heterozygosity of 6% seems small, reductions in heterozygosity of similar magnitudes have been shown to decrease viability and fecundity in some individuals (Ralls and Ballou, 1983). Finally, populations of wild turkeys in the eastern United States have been established with more

individuals than at PWMA (Leberg, 1991; Leberg et al., 1994), perhaps contributing to the greater success of reintroduced populations east of the Mississippi River.

The most obvious explanation for differences between our study and those of Leberg (1991) and Leberg et al. (1994), who found virtually no genetic variation within turkey populations, relates to the unique advantages and limitations of the different genetic approaches used (Murphy et al., 1990; Hanotte et al, 1992; Leberg 1996; Rassmann et al., 1996). Our results cannot be compared directly with those of Leberg (1991) and Leberg et al. (1994) because different populations with different demographic histories were analyzed. A critical next step is to use multi-locus DNA fingerprinting to analyze populations examined by Leberg (1991) and Leberg et al. (1994) and to use protein electrophoresis to examine individuals from the PWMA population. With these data, we may determine if consistent patterns of genetic variability are apparent and thus provide more insight into the effect that reintroductions have on heterozygosity and population viability.

Leberg (1993) proposed that allelic diversity is more important in conservation than heterozygosity levels. He demonstrated that allelic diversity of founders affected success of resulting populations of mosquitofish (Gambusia holbrooki) more than either founder heterozygosity or the number of stocks from which founders were taken. In our study, average allelic diversity within flocks for minisatellite and microsatellite loci was 4.17 and 4.70, respectively. These levels are similar to the mean number of alleles per locus reported within a healthy, self-sustaining population of clapper rails at the Salton Sea (5.5; Fleischer et al., 1995) and within three species of swans (6.5 - 9.2; Meng et al., 1990). However, considerably lower levels of mean allelic diversity have been reported for declining coastal populations of clapper rails (1.9 - 2.6; Fleischer et al., 1995) and captive populations of endangered Hawaiian nene (Branta sandvicensis; 2.0 - 2.3; Rave et al., 1993). Although estimates of allelic diversity do not exist for turkeys in Arkansas and Missouri for the period in which birds were translocated to eastern Oklahoma, or

other populations of wild turkeys, we speculate that the few individuals founding the PWMA population (nine individuals) lacked much of the allelic diversity present in historic populations prior to decades of human exploitation.

Because hypervariable segments of the genome revealed via multi-locus DNA fingerprinting often are extrapolated to provide an estimate of overall genomic heterozygosity, it is possible, although unlikely, that genetic attributes associated with fitness, fecundity, and viability do not contain the same level of variability. This contradicts findings of other researchers (Brock and White, 1992; Hanotte et al., 1992; Longmire et al., 1992; Haig et al., 1993; Young et al., 1998) that low levels of band sharing and high heterozygosity are correlated positively with robust fitness indices (high fecundity and viability).

CONCLUSION--Eastern wild turkeys have been reintroduced successfully to many parts of their historic range (Leberg, 1996), but the effect of reintroductions on genetic structure of resulting populations is unclear and may vary among regions. Although Leberg et al. (1994) suggested that reintroductions have no negative effect on viability of wild turkey populations, they conceded that they were able to sample only those sites where reintroductions resulted in viable populations. Wild turkeys in southeastern Oklahoma decreased 77% from 1980 to 1994. Results from multi-locus DNA fingerprint analysis indicate that turkeys at PWMA contain levels of similarity and heterozygosity not unlike other outbred avian populations and, therefore, do not appear to suffer from inbreeding depression. Moreover, high heterozygosity, low similarity, and no significant correlation between similarity and geographic distance all suggest frequent dispersal of females among flocks in and around PWMA.

When reintroductions fail to produce a self-sustainable population, wildlife managers often release more individuals in subsequent years (Leberg et al., 1994). We do not recommend further reintroductions of eastern wild turkeys to southeastern

Oklahoma. History has shown that with proper protection, habitat improvement, and public awareness, wild turkeys can proliferate to reach substantial numbers from only a small number of individuals (Dickson, 1992). The advantage of fostering existing populations (with adequate genetic diversity) in times of decline is that adaptive alleles are maintained in the future gene pool. The overall decline of reintroduced turkeys in southeastern Oklahoma may reflect their natural progression toward local habitat adaptation by losing poorly adapted genotypes from the gene pool. If further reintroduction is deemed necessary, it is imperative that the location and environment of source populations be evaluated, especially considering that southeastern Oklahoma is the western-most margin of eastern wild turkey distribution. Most populations are adapted to their local environment and these adaptations may not serve a long-term benefit for the fitness of animals reintroduced to foreign habitats and their future progeny (Dobzhansky, 1948; Carson, 1983; Templeton, 1986; Leberg et al., 1994).

Given the high genetic diversity observed, the decline of the wild turkey in southeastern Oklahoma may be due to biotic and abiotic factors not accounted for in this study. Research examining multiple demographic influences on this population is nearing completion and an evaluation of these data in conjunction with genetic data from this study may aid area managers in their attempt to produce sustainable populations of eastern wild turkeys in southeastern Oklahoma and other regions.

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Table 1--Descriptive statistics for genetic variation within eight flocks of eastern wild turkey (Meleagris gallopavo silvestris) from southeastern Oklahoma. n = individuals sampled per flock; $(GT)_n$ = microsatellite probe; pV47-2 = minisatellite probe.

		Bands/individual		Similarity		Number of loci		Heterozygosity		Alleles/locus	
Flock	n	$(GT)_n$	pV47-2	$(GT)_n$	pV47-2	$(GT)_n$	pV47-2	$(GT)_n$	pV47-2	$(GT)_n$	pV47-2
1	11	17.82	22.00	0.34	0.41	9.86	12.53	0.80	0.75	6.06	5.42
2	4	18.00	20.50	0.34	0.43	9.93	12.90	0.81	0.59	4.53	3.41
3	6	17.33	16.83	0.37	0.34	9.72	9.65	0.78	0.74	4.94	5.07
4	4	17.75	21.00	0.32	0.45	10.03	12.62	0.77	0.66	4.48	3.56
5	10	17.40	17.20	0.36	0.37	9.77	9.67	0.78	0.78	6.04	5.89
6	2	19.00	27.25	0.47	0.57	13.97	21.21	0.36	0.34	2.08	2.03
7	13	18.31	22.85	0.37	0.47	10.29	13.71	0.78	0.67	6.12	4.74
8	4	21.25	21.25	0.45	0.47	12.74	13.48	0.67	0.58	3.38	3.26
Mean		18.36	21.11	0.38	0.44	10.79	13.22	0.72	0.64	4.70	4.17

Table 2--Pairwise similarity[1] among flocks of eastern wild turkey (Meleagris gallopavo silvestris) in southeastern Oklahoma based on Hinf I/$(GT)_n$ (below diagonal) and Hae III/pV47-2 (above diagonal) multi-locus DNA fingerprinting.

Flock	1	2	3	4	5	6	7	8
1		0.39	0.43	0.47	0.48	0.47	0.45	0.44
2	0.38		0.45	0.43	0.47	0.41	0.41	0.41
3	0.37	0.38		0.46	0.47	0.44	0.47	0.46
4	0.38	0.41	0.39		0.47	0.48	0.48	0.45
5	0.37	0.37	0.40	0.36		0.49	0.47	0.48
6	0.39	0.40	0.41	0.35	0.33		0.46	0.51
7	0.34	0.43	0.42	0.39	0.38	0.36		0.45
8	0.41	0.40	0.39	0.36	0.45	0.43	0.37	

[1] Comparisons made from flock representatives adjacent on a single gel.

Chapter 3

CHARACTERIZATION OF THE MHC CLASS IIß$_1$ LOCUS IN THE EASTERN WILD TURKEY (Meleagris gallopavo silvestris)

Characterization of the MHC Class IIß$_1$ locus in the Eastern Wild Turkey (Meleagris gallopavo silvestris)

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Summary

The highly polymorphic second exon of the Major Histocompatibility Complex (MHC) Class IIβ_1 locus was characterized from an Oklahoma population of Eastern Wild Turkeys (*Meleagris gallopavo silvestris*). Five Class IIβ_1 fragments were cloned and sequenced from five turkeys. The Class IIβ_1 sequences exhibited differences in both length (216 and 180 bp) and composition. Of the 180 bp common to all five alleles, 20 percent of nucleotide and 26 percent of amino acid positions were polymorphic. Nonsynonymous substitutions occurred as frequently as synonymous substitutions within the paralogous regions of Class IIβ_1 exons that were sequenced ($d_N = 0.1538 \pm 0.0182$; $d_S = 0.2229 \pm 0.0352$: $t_\infty = -1.7408$; $P = 0.0957$).

Key Words: allele, locus, MHC, polymorphism, wild turkey

The MHC is an ancient group of genes known to regulate immune response in vertebrates. Within the MHC, Class II genes contain highly polymorphic exons that encode antigen binding sites (ABS). Because the ABS is the initial point-of-contact for recognizing, binding, and ultimately presenting foreign pathogens to T-cells for destruction, sequence diversity within this region is functionally important, and can determine individual ability to resist parasites and disease (Hughes 1989). Some MHC genotypes have been positively correlated with fitness, parasite loads, development of secondary sexual characters, and sexual selection (for review, see Finch & Rose 1995). In avian Class II molecules, the ABS that has exhibited the greatest amount of polymorphism is the second exon of the β chain (β_1) (Hughes *et al.* 1994).

The high level of variability and trans-species mode of evolution observed among some MHC alleles (Klein 1987; Klein *et al.* 1993; Van Den Bussche *et al.* 1999) recently has made the MHC a popular genetic marker for addressing many evolutionary and biological questions. Yet to date, characterization of the MHC among birds has been

limited primarily to the domestic chicken (*Gallus gallus domesticus*), Ring-necked Pheasant (*Phasianus colchicus*) and a few Old and New World passerines (Edwards *et al.* 1995; Vincek *et al.* 1997). Although restriction fragment-length polymorphism (RFLP) analysis of MHC fragments within lines of domestic poultry has shown that certain MHC haplotypes are associated with growth, reproduction, and disease resistance (Bacon 1987; Nestor *et al.* 1996), the sequences of MHC Class IIβ_1 alleles specific to North American galliforms have yet to be fully characterized. In addition to the economic benefits that could be realized by the poultry industry, the DNA sequence of MHC Class IIβ_1 alleles in turkeys could be examined by researchers to help reconstruct the evolutionary histor y of galliforms as well as genes within the MHC itself. Therefore, our objective was to characterize the MHC Class IIβ_1 locus in Eastern Wild Turkeys.

Tissue samples were obtained through spring hunter harvest and direct capture of individuals at the Pushmataha Wildlife Management Area in Pushmataha Co., Oklahoma, during the period of December 1995 - May 1997. Either 1 gram of liver tissue or three drops of blood were placed in 5 ml of lysis buffer for DNA isolation following the protocol of Longmire *et al.* (1997). Using primers 471 (5'-GAGTGCC ACTACCTGAACGGCACCGAGCGG-3') and DR1 (5'-GCTCCTCTGCACCGTGA AGGA-3'), designed from Class IIβ_1 sequences of Ring-necked Pheasants (Wittzell *et al.* 1994), orthologous fragments of DNA comprising the second exon of the MHC Class IIβ_1 locus in turkeys were amplified via Polymerase Chain Reaction (PCR) with a GeneAmp PCR System 9600 thermocycler (Perkin-Elmer). Reaction conditions to amplify 400-600 ng of genomic DNA in 50 µl total volume were: 0.24 mM deoxynucleotides, 1.0 unit of *Taq* DNA polymerase, 0.78 µM primers, and 2.0 mM $MgCl_2$. The PCR thermal profile involved denaturation at 94°C for 3 min, followed by 30 cycles of 94°C, 68°C, and 72°C, with each temperature held 45 sec. Finally, amplicons were allowed to fully extend at 72°C for 30 min.

Amplicons were cleaned using the Wizard PCR Prep DNA Purification System (Promega), and 1 µl of cleaned DNA was used as template to reamplify exon 2 with an upstream primer internal to 471, β44 (5'-ACCCAGCAGGTGAGGCATGTG-3') (Wittzell *et al.* 1994), and DR1. Products verified to be sufficiently concentrated and of appropriate size through screening on 0.8% agarose gels stained with ethidium bromide were cleaned and used as template for radioactive amplification of the Class IIß$_1$ exon using primers β44 and DR1. All PCR conditions remained the same as described before with the exception that deoxynucleotides were reduced to 0.12 mM and 1.0 µCi α^{32}P-dCTP was added for incorporation. Radioactive amplicons were subjected to single-stranded conformation polymorphism analysis (SSCP; Orita *et al.* 1989a, 1989b) whereby PCR products were electrophoresed through a 5% nondenaturing acrylamide gel (acrylamide: bis-acrylamide = 49:1) containing 10% glycerol for approximately 18 hrs at 300 volts, 3 watts, and 10 mAMPS, with a fan cooling the surface of the gel. Conformationally segregated strands were visualized via autoradiography following 24 hrs film exposure at -80°C.

Five unique conformations were cloned using the pGEM-T cloning system (Promega). Inserts of 10-25 recombinant clones for each selected individual were radioactively amplified using reaction conditions and thermal profile described above. Amplicons from cloned inserts were run beside the original radioactively amplified samples for SSCP comparison. Cloned fragments whose migration matched that of the individual were reamplified (without ^{32}P) with primers β44 and DR1, returning dNTP concentration to 0.24 mM. Following cleaning, products were sequenced bidirectionally with a Perkin-Elmer Applied Biosystems 377 automated sequencer. Sequenced MHC Class IIβ_1 alleles were aligned (CLUSTAL W; Thompson *et al.* 1994) and nucleotide and amino acid distance, as well as frequencies of synonymous (d_S) and non-synonymous (d_N) substitutions (Nei & Gojobori 1986) were calculated using the computer program MEGA (Kumar *et al.* 1993).

Following SSCP screening, DNA sequence data revealed five unique Class IIβ_1 sequences from the Eastern Wild Turkey (GenBank accession numbers XXXXXX-XXXXXX). Among these sequences, two distinct size categories were observed, with lengths being 216 bp and 180 bp, respectively. The three long alleles were designated *MhcMega-DAB1* following the nomenclature proposed by Klein *et al.* (1990). We chose not to officially name the shorter alleles at this time, and for the purposes of this publication will refer to them as *Unknown alleles 1 and 2*. Despite the difference in size, the two allele types aligned well, and the *Unknown alleles* appeared to be truncated forms of the alleles from the *Mega-DAB1* locus (Fig. 1). This finding suggests that the *Unknown alleles* may represent a paralogous locus to the *Mega-DAB1* locus, and may be part of a pseudogene formed through a distant duplication event. Although each individual selected for sequencing exhibited at least three clones with identical inserts, it is possible that the *Unknown alleles* are artifacts due to PCR synthesis errors. Because we were unable to obtain more individuals and more clones containing alleles of this type, we withheld entering the sequences of *Unknown alleles 1 and 2* into GenBank.

Overall, 20 percent of nucleotide and 26 percent of amino acid positions were polymorphic among characters common to the five unique alleles. Nonsynonymous substitutions occurred at an equal frequency as synonymous substitutions within the paralogous regions of Class IIβ_1 exons that were sequenced ($d_N = 0.1538 \pm 0.0182$; $d_S = 0.2229 \pm 0.0352$: $t_\infty = -1.7408$; $P = 0.0957$). By removing the two short alleles, (*Unknown alleles 1 and 2*) from the analyses, which were possibly amplified from a pseudogene or PCR artifact, we were able to fully represent all characters from the longer the *DAB1* fragments. Secondary comparison among *DAB1* alleles revealed mean nucleotide distance, corrected for multiple substitutions under the Jukes-Cantor model (Jukes & Cantor 1969), to be 0.1789 ± 0.0824, whereas mean amino acid distance was 0.2207 ± 0.0892. Nonsynonymous substitutions were statistically equal to synonymous

substitutions at the *DAB1* locus also ($d_N = 0.1291 \pm 0.0554$; $d_S = 0.2316 \pm 0.1158$: $t_\infty = -0.7984$; $P = 0.2347$).

It has been suggested that as research into the MHC characterization of birds continues, particularly in galliforms, more avian Class II genes will be discovered (Wittzell *et al.* 1994). Our findings mirror those of previous researchers in that we were only able to identify two types of Class IIβ_1 sequences (Wittzell *et al.* 1999). Until more alleles can be characterized in galliform species and Mendelian inheritance studied through breeding experiments, it is not possible to confidently assign these potential alleles to a specific locus. However, throughout our study, efforts to screen and isolate single alleles were impeded by non-target amplifications and what appeared to be inconsistent amplification of additional loci, even when using every likely primer pair combination developed from birds (Edwards *et al.* 1995; Wittzell *et al.* 1994). SSCP screening analysis often revealed more than six equally dark bands per individual, indicating up to three different alleles were amplified. Although this may lend indirect support for the presence of yet-to-be discovered functional avian MHC Class II genes, it also may be an artifact of technical problems associated with amplification of paralogous sequences. Conserved primers for nuclear genes are likely to be conserved in paralogous pseudogenes as well, leading to amplification of pseudogenes and other paralogs (Moritz & Hillis 1996). Because pseudogenes frequently are created from retroposition of processed mRNA's, and are therefore shorter than their functional paralogs, they can often be identified by a lack of introns and the regulatory sequences necessary for expression. Because our research targeted only the internal portion of a exon, we cannot determine what, if any, regulatory sequences are lacking. We recommend, as did our predecessors (Edwards *et al.* 1995), that future research into the number of Class II loci in birds rely on the large scale characterization of continuous segments of DNA via cloning and sequencing.

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Figure 1. Nucleotide sequences of MHC Class IIβ_1 alleles isolated from five Eastern Wild Turkeys (*Meleagris gallopavo silvestris*).

```
                          10         20         30         40         50         60
MhcMega-DAB1*01  CACTACCTGA ACGGCACCGA GCGGGTGAGG TTGGTGGACA GGTATTTCTA CAACCGACAG
MhcMega-DAB1*02  ...???.... .......... .........A ..T..T..G. ..C.AA.... T....AG...
MhcMega-DAB1*03  .......... .......... .......... .......... .......... ..........
(Unknown allele 1) ---------- ---------- ---------- ------C... ..T.C..... C....G....
(Unknown allele 2) ---------- ---------- ---------- ------GT.. ..C.A..... ..........

                          70         80         90        100        110        120
MhcMega-DAB1*01  CAGTTCACGC ACTTCGACAG CGACGTGGGG AAATATGTGG TCGATACGCC GCTGGGAGAG
MhcMega-DAB1*02  ...ACAT... .......... .......... .....C.... C....T.A.. T....AT..A
MhcMega-DAB1*03  .G....C... .......... .......... .......... .......... ..........
(Unknown allele 1) .A.TACG... .......... .......... ....TT.... .....A.... .....G....
(Unknown allele 2) ....T.A... .......... .......... ....A..... .......G.. ......A..G

                         130        140        150        160        170        180
MhcMega-DAB1*01  CCGACAGCTG AGTACTGGAA CAGCGACACC GAGTTTATGG AAATAAAAAG GAATGAGGTG
MhcMega-DAB1*02  TATCAG.T.. .ACT.............. C...A..... .GTAC.G.C. .GG.......
MhcMega-DAB1*03  .......... .......... .......... .......... .......... ..........
(Unknown allele 1) CCG..A.C.. .GTA.........A..... G..AT.C... ..GG.CT... ..........
(Unknown allele 2) .......... .....C........G..... C..TA.A... ..TA.A.... .....C....

                         190        200        210    216
MhcMega-DAB1*01  GACACGTTCT GCCGGCACAA CTATGGGGTG TTTGAG
MhcMega-DAB1*02  ....G..A.. ....A..... ..TC...... ......
MhcMega-DAB1*03  .......... .......... ..A....... ......
(Unknown allele 1) .......T.. ....G..... .........T GGA...
(Unknown allele 2) ....C..... ....A..... .........G ......
```

() These Class IIβ$_1$ alleles may be partial alleles or nontarget amplifications from pseudogenes or other paralogous loci.

VITA

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