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IDENTIFICATION OF SEGREGATING HAPLOTYPES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN CRANES[1]

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The major histocompatibility complex (Mhc) is thought to exist in all vertebrate species. In most species, a high degree of polymorphism is maintained in this region at multiple loci which encode molecules that serve in the presentation of foreign antigens to T lymphocytes (Klein 1986, Miller 1991). In this investigation, serological and recombinant DNA techniques are being used to investigate genetic diversity at the Mhc in several species of cranes, to investigate questions of paternity in some instances, and to develop practical methods for determining Mhc haplotypes. Blood samples are provided by the Patuxent Wildlife Research Center (PWRC), Laurel, Maryland, and the International Crane Foundation (ICF), Baraboo, Wisconsin.

Appropriately absorbed chicken sera specific for chicken Mhc class I (B-F) and Jarvi 2 class IV (B-G) antigens, chicken anti-crane antisera and crane anti-crane antisera (produced in Florida sandhill cranes [*Grus canadensis pratensis*] at the PWRC) were used to detect segregating Mhc antigens in crane species, including sandhill, sarus *(G. antigone)*, Siberian (*G. leucogeranus*), and whooping (*G. americana*) cranes. Analysis of sandhill crane families with these reagents revealed a minimum of 15 Mhc haplotypes among 38 individuals tested. The sandhill crane antisera were found to reveal polymorphisms in several other species that are presumably due to segregating Mhc haplotypes. For example, among 65 captive whooping cranes 9 distinct patterns emerged utilizing 7 of these antisera. These patterns in conjunction with additional family data suggest a minimum of 8 segregating Mhc haplotypes among these birds.

To develop DNA-based typing methods, we prepared a genomic library from whooping crane DNA. From this library we isolated clones cross-hybridizing with chicken Mhc *B-G* probes and verified through nucleotide sequencing that these clones contain Mhc *B-G* genes. We found that a 1.5-kb subcloned fragment, a portion entirely within a single *B-G* gene, is a probe suitable for revealing restriction fragment length polymorphisms (RFLP) in all 15 species of cranes. We are presently carrying out detailed RFLP analyses of captive whooping, sandhill, Siberian, and sarus cranes by using this and additional whooping crane Mhc *B-G* probes.

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