POPULATION GENETIC STRUCTURE OF BIRDS

INTER- AND INTRAPOPULATION ANALYSIS OF BIRD SPECIES:

RESULTS FROM VNTR AND MHC GENETIC MARKERS

By

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CHAPTER ONE: GENERAL INTRODUCTION

In this thesis, I explored the use of DNA-based genetic markers to assess genetic structure in populations of wild birds. Until recently, most research in this area has used allozyme markers (Aquadro and Avise 1982; Barrowclough 1983). However, this approach has limitations for studies of intraspecific variation because such markers lack variability at this level. Recent advances in DNA technology have provided more accurate and sensitive sets of markers for studies of variation at the population level, such as, mitochondrial DNA sequence data and multi-locus Variable Number of Tandem Repeat (VNTR) loci markers (Nakamura et al. 1987). In addition, the study of avian DNA markers is particularly feasible, since large amounts of DNA can be obtained from a small sample of blood with nucleated erythrocytes, making DNA extraction a rapid and economical process.

Assessing population genetic structure in birds is of great interest to behavioural ecologists because of the insights into the social behaviour of organisms that can be gained from such work. For example, genetic analysis of a population can address questions pertaining to mating systems and social structure. Various mating systems, where some

individuals produce more offspring as a result of dominance, polyandry and polygyny, are predicted to reduce genetic variability in the population. Overlapping generations also increase the chance of matings between parent and offspring, thus contributing to loss of genetic variability. Population genetic analysis can test these ideas. Parentage analysis can also provide essential information about reproductive behaviours. For example, extra-pair fertilizations, polyandry, polygyny, and egg dumping are all behaviours that can be detected using genetic methods (Rockwell and Barrowclough 1987).

One area of my research assesses the genetic structure of two populations of the Tasmanian native hens (*Tribonyx mortierii*) through the use of two types of highly polymorphic loci, hypervariable minisatellite loci (Jeffreys et al. 1985b) and Major Histocompatibility Complex (MHC) loci. Much can be learned about the evolution of behaviour, history and probable health status of this species by comparing the genetic structure within family groups and between populations. The second area of my research assesses the degree of homology between certain chicken MHC loci and similar loci in a wide variety of bird species. In particular, two chicken MHC probes (B-LβII and F10) were hybridized to nine different species of birds and the degree of homology assessed. In addition, levels of variation in the MHC loci in two species,

the European cuckoo (*Cuculus canorus*) and the brown-headed cowbird (*Molothrus ater*), were assessed in more detail. This will aid in future research in avian genetics where assessment of variation at a loci linked to fitness such as the MHC is useful.

The Study Populations

Most of the research in my study focuses on the Tasmanian native hen which is a large, flightless gallinule, endemic to Tasmania. It is a diurnal species, spending most of its day feeding on fine grass and herbage in open fields adjacent to marshlands. Individuals are aggressive defending a territory and they form large social groups consisting of between 3 to 17 individuals. An extensive study into the ecology, behaviour and population structure of this species was carried out by Ridpath (1972a,b,c) in the late 1960s and again in the early 1990s by Goldizen et al. (1993).

Tasmania is an island located south of Australia. It is a mountainous island and hens are distributed along the northern coastline below 700m in elevation through the eastern portion of the island. The two populations of hens examined in this study were chosen for their unique histories. The first population is located on a small island to the east of mainland Tasmania in Maria Island National Park. A population bottleneck due to a founding event was generated in

1965, when four birds were introduced by human intervention to the island. Previous to this introduction, no birds were present on Maria Island. This created an ideal situation for studying the genetic implications of inbreeding. The population has since grown to approximately 300 individuals and appears to be stable (Goldizen et al. 1993). The small population size in the early stages of growth likely forced matings between close relatives, which may have led to high levels of genetic homozygosity in the population. When this occurs, a population may experience inbreeding depression (Packer 1979), expressed as an increase in juvenile mortality (De Bois et al. 1990; O'Brien et al. 1985; Ralls et al. 1988), morphological asymmetry (Wayne et al. 1986a, b), an increase in susceptibility to pathogens (Wayne et al. 1986b) and infertility (O'Brien et al. 1985; Wildt et al.1987).

The second population is located on the eastern coastline of Tasmania in a cow pasture near the town of Geeveston. The study population consisted of 75 banded individuals in August of 1989 and was part of a large continuous population inhabiting a river valley. A population of this size and geographical location is typical of one found on the mainland of Tasmania. One goal of my research was to study the effects of population bottlenecks on the genetic structure of the Tasmanian native hen, by comparing levels of variation at different DNA-based marker loci in the Maria

Island and Geeveston populations.

Young Tasmanian native hens leave their family unit at approximately one year of age and disperse in search of a mate (Ridpath 1972). Due to their inability to fly and various natural barriers (mountains, streams and large forests), dispersal can be restricted. Ridpath (1972) found that Tasmanian native hens dispersed an average of two miles from their natal territory. In general, young female birds are the dispersing sex in most bird species (Greenwood 1980). For example, detailed studies on the dispersal patterns of the Florida scrub jay (Woolfenden 1975; Woolfenden & Fitzpatrick 1978) and the great tit (Greenwood et al. 1978) have supported the idea that birds have female-biased dispersal patterns. However, there are exceptions to the rule since males of the lesser snow goose tend to disperse more widely than females (Cooke et al. 1975). I used genetic data to determine which sex, the male or the female, disperses greater distances in Tasmanian native hens.

As mentioned earlier, these birds live in large social groups which may consist of one or more males and females and the young of two breeding seasons. Their mating behaviour encompasses a wide range of mating systems, such as polyandry, polygyny and polygynandry (Ridpath 1972; Goldizen et al. 1993). When two males join a female and help raise the offspring the mating system is referred to as cooperative

polyandry (Faaborg 1981).

Understanding how a polyandrous mating system would evolve and be maintained is of particular interest to behavioral ecologists. In this type of mating system the female has the burden of producing eggs and raising the young while males are only involved in the raising of the offspring. The question arises as to what advantage two males in a group would gain by both mating with a single female rather than forming a monogamous pair with a single female.

One possibility is that if the two males were in fact first degree relatives (brothers or father/son), then kin selection combined with the advantages of group living could explain why two males were willing to 'share' a mate (Maynard Smaith and Ridpath 1972). Ridpath (1972) had some observational data suggesting that paired males in groups were in fact brothers, but there has been no genetic analysis of this possibility ever conducted. In this study, Ridpath's (1972) hypothesis that related males join an unrelated female to form a mating group was tested through genetic analysis.

The second area of my research attempts to assess the degree of MHC loci homology in a number of species using samples from a taxonomically broad range of bird species. These include, the mallard duck (*Anas platyrhynchos*), Swainson's thrush (*Catharus ustulatus*), yellow warbler (*Dendroica petechia*), white-throated sparrow (*Zonotrichia*

albicolis), brown-headed cowbird (*Molothrus ater*), the common cuckoo (*Cuculus canorus*), the trumpeter swan (*Cygnus buccinator*), the Tasmanian native hen (*Tribonyx morteirii*) and the pukeko (*Porphyrio porphyrio*). In addition, I attempted to assess variation at the population level for two of these species, the common cuckoo and the brown-headed cowbird.

Genetic Markers

The markers I used to assess the genetic structure of the Tasmanian native hen at a population level and then within breeding groups were the hypervariable minisatellites (Jeffreys et al. 1985b) or VNTR loci. These regions are the most variable loci found in the genome with each locus consisting of a series of repeated DNA (17-80 bp) each which have a common 'core' sequence (Jeffreys et al. 1985b). Polymorphisms may arise due to unequal crossover events between tandem repeats of DNA or replication slippage (Jeffreys et al. 1988). A variety of multilocus DNA probes have been developed containing these 'core' sequences, including Jeffreys 33.15 and 33.6 (Jeffreys et al. 1985b), Per (Shin et al. 1985), 3'HVR (Fowler et al. 1988) and M13 (Vassart et al. 1987). A DNA fingerprint or multi-locus VNTR profile can be generated through Southern blotting (Southern 1975) and radioactivly labelling with a minisatellite probe.

This technique known as DNA 'fingerprinting' or profiling has many applications including paternity testing (Helminen et al. 1988), forensics (Gill et al. 1985), immigration cases (Jeffreys 1985b) and analysis of population structure (Faulkes et al. 1990; Reeve et al. 1990; Haig et al. 1993).

The genetic profiles, which are expressed as bands on a DNA fingerprint are inherited in a Mendelian fashion (Jeffreys et al. 1985b) and any band in an offspring can be traced back to either parent. Thus, approximately 50% of the bands should come from one parent and the other 50% from the other parent. The degree of relatedness between individuals can thus be assessed from the DNA fingerprint through the degree of band sharing. This assessment becomes complicated when relatedness between individuals is less than first degree relatives due to varying amounts of background levels of band sharing (Lynch 1988). Inbreeding as a result of a population bottleneck can elevate background band sharing values by fixing certain alleles. This has been demonstrated in mice (Jeffreys 1987), chickens (Kuhnlein et al. 1990) and the eusocial naked mole-rat (Reeve et al. 1990). Therefore, I will use DNA fingerprinting in this study, to detect the level of inbreeding in the populations of Tasmanian native hens under study, as well as assess the amount of genetic similarity between certain individuals in each population and within each social group.

The Major Histocompatibility Complex (MHC) is another highly polymorphic gene family for which probes from particular locus in a bird (chicken) are available. For this part of my research I examined the amount of DNA homology that nine species of birds have, to two chicken MHC probes from a Class I and Class II locus respectively. Then, to gain some estimate of levels of intraspecific variability in these loci, I examined variation in these regions in population samples of three species.

An MHC locus or equivalent has been found in all vertebrate species studied thus far including various primates, rodents, carnivores, ungulates and reptiles and also from the chicken, rabbit, carp and the clawed toad (Klein 1986). The MHC plays a major role in the immune system. It is made up of a variety of genes of which each have a specific function in the bodies defence system. The two most highly studied are the Class I and II regions of the MHC locus. The general role of the Class I and II genes is to produce cell surface glycoproteins which present antigen to various T lymphocytes (Klien 1986).

The second MHC locus discovered was that of the domestic chicken and was named the B complex. This complex encodes for at least three polymorphic cell surface molecules: B-F (Class I), B-L (Class II) and B-G (Class IV). In chickens, the B-F and B-L perform the same function as Class I and II

3). Information generated from this study will, in general, suggest whether such probes will be useful for assessing levels of variation in these immunologically related loci in a wide variety of birds.

Overall, the use of two highly polymorphic loci, the VNTR and the MHC, will provide information about the genetic structure of a number of avian species. At a population level it will show how genetically similar or different the members of a population are to one another and give insight into the levels of inbreeding. The VNTR loci data will also be used to examine the genetic structure of individuals at a group level and reveal relationships within a family unit. The use of MHC markers provides information on the homology of the MHC loci in a wide range of bird species and provides an estimate of the levels of variation in these loci in populations of particular species.

CHAPTER TWO

EXAMINATION OF THE GENETIC STRUCTURE OF TASMANIAN NATIVE HEN (*Tribonyx mortierii*) POPULATIONS: INTER- AND INTRAPOPULATION COMPARISONS USING MULTI-LOCUS VNTR AND MHC DNA PROFILES

ABSTRACT

I used two nuclear DNA-based genetic systems to study
the genetic structure of a flightless bird, the Tasmanian
native hen (*Tribonyx mortierii*), at a population level and
then within breeding groups in the populations.   The
populations under study were located on Maria Island off the
eastern coast of Tasmania and near Geeveston on the mainland.
At a population level, the degree of genetic variability
detected using hypervariable minisatellite markers indicated
high levels of inbreeding in both populations of *T. mortierii*,
with band sharing coefficients (BSC) ranging from 0.582 to
0.728.   The population on Maria Island however, showed
significantly higher ($p < 0.0001$) BSCs among adults as compared
to the Geeveston population. This difference was attributed
to a population bottleneck which occurred on the island in
1965 and the inbreeding which resulted in subsequent
generations. Within each population, the degree of genetic
variability among members of the same sex revealed females as
the dispersing sex, since they showed significantly lower BSCs
than males. At a group level, within each social group in the
Maria Island population, the BSCs between adult males were
statistically indistinguishable from that of siblings. This
suggests that adult males in polyandrous trios are brothers or

possibly fathers and sons. In contrast, analysis from the Geeveston population did not support this type of relationship between the adult males.

A second marker, the Major Histocompatibility Complex, also revealed little variation in both populations as compared to other vertebrates. This locus has been associated with susceptibility to pathogens and thus reduced variation at this locus may increase the susceptibility of birds in these populations to diseases.

Overall, this study has shown how polymorphic DNA markers can be used to assess the genetic structure of bird species at a population and a breeding group level. Such information can be useful in interpreting evolutionary processes in this species especially when combined with behavioural observations.

INTRODUCTION

Assessing the genetic structure of populations of birds is of particular interest to behavioural ecologists and evolutionary biologists, for the information it provides pertaining to their mating systems and social structure. For example, reduced genetic variability within a population could suggest matings between close relatives and/or unusual mating systems such as dominance and polygyny. Also, the degree of genetic similarity between same-sex members in a population can predict which sex on average disperses greater distances. In addition, the degree of genetic similarity between any two individuals will provide an estimate of the degree of relatedness. In particular, parentage analysis will reveal behaviours such as extra pair copulations, cooperative breeding, polygyny, polyandry and egg dumping.

In the past few years, new techniques have become available for assessing population structure in birds. In particular, DNA analysis, which focuses on highly polymorphic regions of the genome, allows sensitive testing for the degree of variability that exists among individuals of a population. In particular, avian genetic research has benefited greatly from this approach since it is relatively easy to extract DNA from their nucleated red blood cells. This approach has

centered on the use of hypervariable minisatellite sequences (Jeffreys et al. 1985b) and Major Histocompatibility Complex (MHC) loci, since these regions are exceptionally variable. Researchers previously had to rely on allozyme techniques which did not detect many variable loci (Aquadro and Avise 1982; Barrowclough 1983), and thus did not give an accurate fine-scale assessment of the level of genetic variability found in a population.

In this study I used DNA-based genetic markers derived from regions scattered throughout the genome, the hypervariable minisatellite locus (Jeffreys 1985b) or the multi-locus Variable Number of Tandem Repeat (VNTR) locus (Nakumara et al. 1987) and the Major Histocompatibility (MHC) locus, to study variation at an inter- and intrapopulation level in the Tasmanian native hen (*Tribonyx mortierii*). VNTR loci are the most variable loci discovered thus far in humans (Nakamura et al. 1987). They consist of a series of repeated sequences of 10 to 80 base pairs. It is this variation in the number of repeated sequences that produces the genetic variation detected using minisatellite probes. Each repeat contains a 'core' sequence which differs for each minisatellite locus (Jeffreys et al. 1985b). Southern blotting and hybridization with a probe containing this 'core' sequence, allows detection of the DNA variation in the form of a DNA fingerprint. A DNA fingerprint consists of a series of

fragments of varying molecular weights in the form of bands on an autoradiograph. Each individual possesses a unique banding pattern except in the case of identical twins. These bands segregate in Mendelian fashion with every band traceable to either parent. Therefore, it is possible to generate band sharing coefficients (BSC), to assess genetic similarity between two individuals (Lynch 1988, 1990, 1991). According to Lynch (1988, 1990, 1991), establishing exact relatedness can only be accomplished between first degree relatives. This is due to the compounding effects of inbreeding which will elevate the background band sharing levels. However, DNA fingerprinting is still a powerful tool in studies in which the goal is to determine genetic similarity, since BSCs have a linear relationship with the degree of relatedness among individuals (Brock and White 1991; Lynch 1988, 1990, 1991).

VNTR loci (Nakumara et al. 1987) have become increasingly important in many areas of research in population biology. For example, at a population level this type of DNA marker has been used to examine interpopulation differences (Gilbert et al. 1990; Hoelzel and Dover 1991; Wayne et al. 1991a,b), population bottlenecks (Packer et al. 1991), population structure (Faulkes et al. 1990; Reeve et al. 1990; Haig et al. 1993), genetic distance between populations (Kuhnlein et al. 1989; Siegel et al. 1992) and population dispersal patterns (Rabenold et al. 1991). Analysis at a

family level have also been carried out in the form of parentage analysis (Burke and Burford 1987; Wetton et al. 1987; Burke et al. 1989; Birkhead et al. 1990; Morton et al. 1990; Rabenold et al. 1990; Westneat 1990; Tegelstorm et al. 1991) and establishing relatedness among individuals (Jones et al. 1991; Packer et al. 1991; Gilbert et al. 1991; Blanchetot 1991; Cummings and Hallett 1990).

A second highly polymorphic system which has been studied, is the MHC locus, which includes genes involved in regulating the immune system. Every animal species studied thus far has a gene complex equivalent to that of the MHC loci in mice (Klein 1986). The only avian MHC loci studied in detail thus far is that of the chicken and it is named the B complex (Guillemot et al. 1989). The B complex is made up of at least three regions, the B-F (Class I), B-L (Class II) and B-G (Class IV). The class I and II genes encode for cell surface glycoproteins which present foreign antigens to T lymphocytes (Guillemot et al. 1989). Many invading antigens (bacteria and viruses) have the ability to change their morphological or genetic structure in ways which render them undetectable to the immune system. Having a highly polymorphic MHC locus is thought to increase the chance of the antigen-presenting cells to recognize foreign antigens (Klein 1986). The African cheetah population which has lost most of its variation at this locus has had an increase in

susceptibility to pathogens (O'Brien et al. 1985). This loss of variation has been attributed to a recent population bottleneck which resulted in inbreeding or matings between close relatives (O'Brien et al. 1985). Inbreeding may also lead to inbreeding depression, which may cause an increase in juvenile mortality (O'Brien et al. 1985 and Ralls et al. 1988; Ballou and Ralls 1982; Van Noordwijk and Scharloo 1980), morphological asymmetry (Wayne 1986a,b) and infertility (O'Brien et al. 1985; Wildt et al. 1987). MHC markers have allowed examination of interpopulation differences although in much less detail (McGuire et al. 1985; Nizetic et al. 1985; Yuhki 1989; Yuhki and O'Brien 1990).

The Tasmanian native hen is a large flightless gallinaceous bird which is endemic to Tasmania and is distributed along the northern and eastern coastlines of the island. My study focused on two populations. One is located near the town of Geeveston on the mainland of Tasmania while the other is found on Maria Island off the eastern coast of the mainland (Fig. 1-1). Two features make an examination of the structure of these populations interesting. First, the histories of the two populations differ in ways that may have altered the degree of genetic variability within each. In 1965, the island population was founded by four birds which were introduced by the Tasmanian Park Service. This event will lead to high levels of inbreeding in the following

generations in comparison to the Geeveston population which was surrounded by other Tasmanian native hens. Thus by comparing the genetic structure of these two populations the effects of different historical events may be detected in their relative levels of variability. Second, birds in both populations exhibit a wide range of mating behaviours, including monogamy, polygyny, polyandry and polygynandry (Ridpath 1972b; Goldizen et al. 1993). Of particular interest is cooperative polyandry where two males join a female to form a mating group and all adults contribute to the raising of the young (Faaborg and Patterson 1981). Other avian species which exhibit cooperative polyandry are the acorn woodpecker (Stacey 1973), Galapagous hawk (Faaborg et al. 1980), dunnock (Davies 1983), dusky moorhen (Garnett 1980) and the pukeko (Craig 1980). In the late 1960s, Ridpath (1972a,b,c) carried out an extensive study on the ecology, behaviour and population structure on a wild population of Tasmanian native hens. One of his major findings was observational data suggesting that a polyandrous trio usually consisted of two brothers and an unrelated female. However, no genetic analysis has ever been attempted to confirm these observations. Analysis of VNTR profiles can estimate the degree of relatedness between individuals in a group to test this hypothesis.

In this study I assess the genetic structure of the Tasmanian native hen at a population level and within each

breeding group using VNTR markers. Information generated from interpopulation comparisons addresses questions about the genetic history of the populations by revealing various levels of inbreeding and by estimating the relative amount of genetic variability in each population. Intrapopulation analyses addresses the evolution of dispersal and polyandrous mating behaviours of these birds by examining the genetic relatedness between individuals within each population and mating group. Also, in this study, I assess the level of genetic variability in MHC-related loci in the two populations of the Tasmanian native hen using probes derived from chicken MHC loci. This information can be used as an estimate of the amount of disease resistance in both populations.

METHODS

Sample Collection: Blood samples were collected by Ann and Alan Goldizen in Tasmania and sent to me for genetic analysis. Adults were captured in traps made of chicken wire. Blood samples (100 μl) were taken from the brachial vein of anaesthetized birds and mixed into lysis buffer (Seutin et al. 1991).

DNA Extraction: Blood and lysis samples (100 μl) were suspended in more 1X lysis buffer (3.5 ml) and rotated slowly overnight at 37°C. Proteinase K was added to the samples in two, 65 unit doses at 12 hour intervals and rotated at 37°C. DNA was extracted with 70% phenol/30% chloroform twice and once with chloroform, then precipitated with one tenth the volume of 0.3M sodium acetate (pH 5.5) and two volumes of 95% ethanol. The DNA was spooled with a hooked glass pipette, washed with 70% ethanol and allowed to air dry for 5 minutes. The DNA was resuspended in approximately 700μl of 1X TNE2 (10 mM Tris-HCl, 10 mM NaCl, 2 mM EDTA) and rotated at 37°C overnight to ensure homogeneity. A TKO 100 Mini-Fluorometer was used to quantify the DNA.

DNA Digestion and Gel Electrophoresis: AluI was the enzyme chosen for the VNTR analysis since it gave the highest amount of variability when probed with the multilocus probes used in this study (see below). For the MHC analysis, I chose the enzyme *HinF*I since it provided the most variable profile when blots of individuals were probed with the two MHC probes (see below). For the digestion, five µg of DNA from each individual sample was digested for 5 hours at 37°C with 10 units of *Alu*I (Pharmacia) and 10 units of *Hinf*I (BRL). The digested DNA, loading dye (bromophenol blue), and 1.24 ng of a molecular size marker (*Hind*III-, *EcoR*I- and *BstE*III- cut lambda DNA) was run on a 0.8% agarose gel made with 0.5x TBE (0.045M Tris-borate, 0.001M EDTA). To generate the VNTR profiles, DNA cut with *Alu*I was run for approximately 54 hours at 65V in a 22 cm long tank. To generate the MHC profiles, the DNA cut with *Hinf*I was run at 65V for approximatly 24 hours in a 20 cm long electrophoresis tank.

Southern Blotting: The gel was soaked in a 0.25N HCl solution for 10 minutes to nick the DNA, then transferred to a denaturing solution (0.6M NaCl; 0.4M NaOH;) for 1 hour, followed by a 1 hour treatment with neutralizing solution (1.5M NaCl; 0.5M Tris-HCl; pH 7.2). Then the gel was placed on a transfer apparatus (Southern 1975) and was blotted overnight onto a PVDF membrane (Immobilon-N ®). The following

day, the membrane was rinsed in 1X SSC, air dried and baked at 80°C for 1 hour.

Choice of DNA Probes: To choose the VNTR probes to be used in this study, I first probed test blots with five VNTR probes: Per, 3'HVR, Jeffreys 33.15 and 33.6 and M13. Per (Shin et al. 1985) and Jeffreys 33.6 (Jeffreys et al. 1985b) sequences were the most homologous to sequences in the Tasmanian native hen DNA since hybridization resulted in sharp, intense banding patterns that were clear and scorable. M13 and Jeffreys 33.15 had cluttered banding profiles and 3'HVR gave a weak signal. Based on these results I chose Per and Jeffreys 33.6 as the VNTR probes to be used in this study. The B-LβII (0.917 kilobases) and F10 (1.284 kilobases) MHC probes, isolated from the chicken, were chosen since these regions of the MHC complex show similarity in a wide range of species (Guillemot et al. 1988, 1989).

Probing: The VNTR blots were prehybridized in a plastic bag with 12 ml of sodium orthophosphate solution (7% SDS [pH 7.2]; 1mM EDTA [ph 8.0]; 1% BSA [fraction V]; 0.25M Na2HPO4 [pH 7.2]) (Westneat et al. 1988) in a 65°C shaking water bath overnight. The two multilocus DNA probes (Per and Jeffreys 33.6) were radioactively labelled with 32P dCTP by random primer extension using an Oligolabelling kit by Pharmacia

(Feinberg and Volgelstein 1983), cetrifuged in a sepharose spin column, added to the solution and then allowed to hybridize overnight at 65°C. Next, the blots were washed (2X SSC; 0.1% SDS) at 65°C for two 30 minute intervals to remove any background radioactivity. The blots and intensifying screen were placed in cassettes for 24 hours with Cronex (Dupont) X-ray film and then on Kodak XAR film for 3 to 20 days, depending on the strength of the signal. Before reprobing, blots were stripped with a solution of 0.4M NaOH for 45 minutes and then neutralized with 0.5% SDS, 0.1X SSC, and 0.2M Tris (pH 7.5) for 45 minutes.

To generate MHC profiles, I used the protocol outlined above with the following exceptions: Inserts of the chicken MHC probes, F10 and B-LβII, were amplified using the polymerase chain reaction (PCR) then were radioactivly labelled through random primer extention (Feinberg and Vogelstein 1983). The probes were hybridized with the blots in 20 mls of a solution consisting of 1.0% SDS, 1.0M NaCl and 10% dextran sulphate and 10 μg/ml of denatured salmon sperm. Blots were washed (0.1% SDS; 1X SSC) two times for 30 minutes at 65°C.

Profile Analysis: Bands on the autoradiograph that were the same relative intensity and within 0.5mm of each other were considered to be the same band. Bands that were approximately

twice the intensity were homozygotes at that allele and were
thus treated as two separate bands (Kuhnlein et al.1990). The
lambda fingerprint cocktail was used as a size reference to
ensure that any difference in lane mobility was accounted for
when comparing band locations (Galbraith et al. 1991). A
chart was then prepared comparing all possible pair-wise
combinations of the birds. The total number of bands for
individuals a and b was designated Na or Nb and the total
number of bands shared between them was designated Nab. The
Band Sharing Coefficient (BSC) was calculated for all pair-
wise comparisons of birds using the equation $D = 2Nab/Na+Nb$
(Lynch 1988). The BSCs were calculated for all unrelated
adults (adults in seperate mating groups) and siblings in the
Maria Island and Geeveston populations for both hypervariable
minisatellite probes (Per and 33.6). BSCs for MHC profiles
were calculated using the same equation for the adults in the
two populations of Tasmanian native hens. All individuals
were blotted and probed twice to eliminate the possibility
that differences in BSCs were a result of blotting or
hybridization differences, which may increase the
detectability of invariant bands or decrease the detectabilty
of variable bands.

Statistical Analysis: The non-parametric Wilcoxson Rank Sum
test (Zar 1984) was chosen to test the various hypotheses

RESULTS

VNTR and MHC Profiles: The two VNTR probes used in this study both hybridized well to the Tasmanian native hen DNA. Examples of VNTR profiles generated using the different probes for the two populations of Tasmanian native hens are shown in Figures 2-2 through 2-5. The band sharing coefficients (BSC) for unrelated adult Tasmanian native hens compared to other vertebrates are shown in Table 2-1. Results show that, on average, BSCs for the Tasmanian native hen populations were typically 2 to 3 times higher than those for some outbred vertebrate species. B-LβII, the class II chicken MHC probe hybridized reasonably well to the DNA and showed some variability but much less in comparison to the DNA fingerprint profiles. The F10 class I MHC probe, however, did not hybridize to the DNA very well but some information could be deduced from the blurred profiles. Figures 2-6 through 2-9 provide examples of MHC profiles for the two populations of Tasmanian native hens. The mean BSCs for the two probes were compared to other studies and are shown in Table 2-2. The BSCs were comparable to other populations which have recently experienced a population bottleneck (*A. jubatus* and *P. leo*) and higher than those which are outbred.

Interpopulation Comparisons through VNTR and MHC Profiles:
The two populations of Tasmanian native hens were compared
with respect to the amount of genetic similarity revealed
through band sharing values (Table 2-3). Average band sharing
coefficients for all pair-wise comparisons between unrelated
adults did not include comparisons among group members, in
order to eliminate the possibility of inflating the background
band sharing values which would occur if the group members
were related. Values for individual comparisons for each
population are shown in Appendices 2-1 and 2-2. The data
showed the Maria island population to have significantly
higher band sharing values than for the Geeveston population
for both Per (Maria=0.682; Geeveston=0.582; $p < 0.0001$) and
Jeffreys 33.6 (Maria=0.728; Geeveston= 0.606;0 $p < 0.0001$)
probes in the VNTR analysis.

Since there was a trend for males within a population
to be more genetically similar (see below) it was necessary to
remove any bias in this comparison that may result from
samples from each population having different numbers of males
and females. On Maria Island more adult males (N=15) than
females (N=11) were sampled as compared to Geeveston where
almost equal samples of males (N=15) and females (N=16) were
sampled. This bias could result in an artificially inflated
overall BSCs for the Maria Island population. To eliminate
possible effects of this bias on the interpopulation

comparison of BSCs, the two populations were broken down by sex and BSCs between populations compared at this level. These results support the original findings. The Maria Island population was again, significantly more similar at the VNTR loci than the Geeveston population for both Per (BSC Maria males=0.681; Geeveeston males=0.604; p=0.0009: BSC Maria females=0.665; Geeveston females=0.559; p=0.004) and Jeffreys 33.6 probes (BSC Maria males=0.759; Geeveston males=0.614; p<0.0001: BSC Maria females=0.688; Geeveston females=0.602; p=0.0035).

The MHC data did statistically support the VNTR data for between population differences in BSCs. BSCs for all pair-wise comparisons of MHC profiles for adults in the two populations are shown in Appendices 2-4 and 2-5. B-LβII and F10 probes for both populations showed difference in band sharing coefficients generated from profiles at those loci, however, these values were virtually indistinguishable at a biological level (Table 2-3). Extremely high MHC band sharing values were found in both populations of Tasmanian native hens.

Intrapopulation Comparisons using VNTR Profiles: The level of genetic similarity can differ between males and females of the same population and this difference within sex can suggest which sex disperses from a local population more than the

other. For example, lower band sharing means less genetic similarity to others in the population which could be due to dispersal from local populations. To see if a difference in genetic similarity existed between the two sexes, the amount of band sharing was compared between adult males from different groups and then between adult females from different groups of the two populations of Tasmanian native hens. Comparisons were only made between non-group members to minimize the possibility of comparing related individuals from the same group. In all comparisons, the female to female band sharing coefficient was lower than the male to male band sharing coefficient (Table 2-4). The data, however, were only significant for two cases, Maria Island birds probed with Jeffreys 33.6 (M/M=0.76; F/F=0.689; p=0.0012) and Geeveston birds probed with Per (M/M=0.604; F/F=0.56; p=0.048). However, there appears to be a trend supporting the idea that the females are the dispersing sex in this species.

Intragroup Comparisons through VNTR Profiles: In three out of four cases the BSCs between the males in a group were significantly higher than those between males and females in a group (Table 2-5 and 2-6). Where the result was non-significant, the mean value for the male to male comparison is still higher than that for the female to male comparison. So far there is a trend that the males within a mating group are

more genetically similar to each other than they are to the females of the group. It is possible that this trend may be an artifact of patterns dispersal described above. The adult males within both populations were previously found to have high levels of band sharing when compared to the females in the population (Table 2-4). If all of the unrelated adult males in a population are more genetically similar, then the males within a group would tend to be more genetically similar as a result. To eliminate this problem, further analysis was carried out by comparing the BSCs between the males of the population with the males of the group (Table 2-7 and 2-8). There were significantly higher levels of genetic similarity found within the males of a group ruling out the possibility that background dispersal patterns were inflating patterns of male to male relatedness within groups.

Analyses were extended to see if, in a polygynous or polygynandrous group where two females belong to the group, the two females were closly related as were the males within a group (see above). The amount of genetic similarity should be greater between the two females than between the females and the males of the group. The results did not support this hypothesis: In all four cases the females BSCs were not significantly different than the male to female BSCs (Table 2-5 and 2-6). It should be noted however, that the sample size for female to female comparisons was extremely small.

Finally, to examine the idea that the males within a mating unit were siblings, I compared the BSCs of group males with that of known siblings based on analyses of parentage carried out by Gibbs et al. (in prep). BSCs for all pair-wise comparisons of siblings in both populations are given in Appendix 2-3. For the Maria Island population there was no significant difference in the BSCs between males within a group and those between known siblings (Table 2-7 and 2-8). However, in the Geeveston population one of the two comparisons of BSCs was significantly different (Jeffreys 33.6; M/M=0.690; Sib=0.833; p = 0.0031) indicating that siblings had higher BSCs than did group males. Therefore the idea that Tasmanian native hen groups are made up of males who are brothers is strongly supported for the Maria Island population but is not supported for the Geeveston population.

DISCUSSION

There are three main findings of my study: First, while both populations of Tasmanian native hens show high levels of genetic similarity within each population, the mean BSCs of adults in the Maria Island population is significantly higher than the Geeveston population, likely as a result of a founder event. Second, analyses of relatedness among adult group members suggests that females tend to disperse more than males. Third, at least with the Maria Island population, the males of a breeding group tend to be first degree relatives.

Interpopulation Comparisons: Maria Island adults showed significantly higher levels of band sharing in their VNTR profiles as compared to adults in the Geeveston population. This pattern was consistent with differences in the histories of each population. The population on Maria Island was initiated by only four birds in 1965 while the Geeveston population is located in a large area of suitable habitat for these birds. However, the Geeveston population unexpectantly had higher BSCs than typically observed for an outbred population (Table 2-2).

The founder effect (Nei et al 1975) is a key factor in reducing genetic variability. Small populations founded by a

few colonists that remain small and are isolated from any gene flow can experience random genetic drift. Genetic drift results in a decline in heterozygosity in successive generations and this process may explain why birds on Maria Island show limited variation in their VNTR profiles as compared to more typically outbred populations.

Loss of genetic variation at certain loci has also been related to a decease in fitness of individuals within population (Ralls et al. 1988; O'Brien et al. 1985). For example, inbreeding causes lethal recessive and other detrimental alleles, which are not common among individuals of an outbred population, to be expressed and this results in high infant mortality (Ralls et al. 1979: O'Brien et al. 1985; Ballou and Ralls 1982; Van Noordwijk and Scharloo 1980) and morphological abnormalities (Wayne 1986a,b). In addition, the MHC locus is responsible for defending the organism against invading pathogens. Reduced variability at specific MHC loci has been attributed to a decline in fitness in the African cheetah population (O'Brien et al. 1985). Polymorphisms are essential to help the immune system keep up with the ever evolving pathogens that constantly invade the body. The Maria Island population had almost no variation at these loci, therefore, these monomorphic birds are possibly in danger if a pathogen invades the population. However, despite the loss of variation in the Tasmanian native hen population

on Maria Island, the population appears to be successful, at least in the short term, having increased its numbers to over 300 individuals in the last 18 years. This population may attribute its success to the founding birds if they were already inbred and had lost their recessive deleterious alleles or if a benign environment exists on the island which has not yet resulted in strong selection pressures acting on this population.

As expected, the VNTR analysis of the Tasmanian native hen population at Geeveston revealed lower levels of genetic similarity when compared to the Maria Island population due to gene flow. However, the BSCs from the Geeveston population were still remarkably high as compared to many outbred populations of vertebrates and in fact were typical of those which characterize highly inbred populations (Table 2-1). There are three possible explanations for this result: First, there was an undocumented population bottleneck in the recent past due to disease, drastic change in weather or fire. The effects of a dramatic reduction in population size would not likely be as devastating to the mainland population as compared to the island population since gene flow could still act to maintain some genetic variation within the population. Second, biological characteristics of these birds and the nature of their habitat may commonly result in inbred populations of this species. Dispersal may

be restricted by their inability to fly, possibly creating small, highly subdivided populations across the mainland. Populations may frequently undergo 'founder-flush' cycles leading to reduced levels of genetic variability. Third, it is possible that the population decline occurred a few months before the samples were obtained (Goldizen et al. 1993), therefore affecting the structure of the population by reducing genetic variability. However, this seems unlikely since the population was well over 75 individuals when samples were taken. This would seem to be large enough to maintain high levels of genetic variation.

The MHC profiles for the Geeveston population also showed little genetic variability. This may provide a causal explaination for why the population reduction in 1989 occurred since Goldizen et al. (1993) attributed the decline to an infectious disease which was affecting neighbouring populations of Tasmanian native hens.

This study has shown how two types of DNA-based genetic markers can be used to determine the genetic structure at a population level in a bird species. It has provided genetic evidence supporting the effects of known historical events such as the founder effect on Maria Island and the population decline at Geeveston on levels of variation in the Tasmanian native hen populations. This type of application is becoming important in conservation biology since information

pertaining to levels of genetic variability in endangered species can enhance captive breeding programs and wildlife management (Hughes 1991). Techniques such as these already have been applied to assess genetic variation in the African cheetah (O'Brien et al. 1985) and Puerto Rican parrot (Brock and White 1991) both of which are endangered species. Both of these species have had their genetic profiles analyzed and individuals who differ in their genetic composition are paired together in hopes of reducing inbreeding depression.

Intrapopulation Analysis: Genetic evidence from the VNTR data indicates that the female Tasmanian native hens disperse more than males. This is consistent with data from other species which show that in general females tend to be the dispersing sex in birds (Greenwood 1980). Factors which may promote this difference in dispersal between the sexes include: Females as the heterogametic sex (Whitney 1976), male dominance (Gauthreaux 1978), population density (Lidicker 1975), and avoidence of inbreeding (Greenwood and Harvey 1976). Greenwood (1980) proposed that philopatry in males is due to resource competition: It easier for a male to aquire a territory in a familiar area. This factor becomes increasingly important when the sex ratio favours females as in the case for the Tasmanian native hen population studied by Ridpath (1972).

I also used VNTR data to examine the possibility that social groups are made up of related males and unrelated females. VNTR analysis showed two results in support of this idea: First, males within each group had the highest BSCs of any other comparison between adults in either population. Second, the BSCs generated from the male to male comparisons were not significantly different from BSCs of presumed siblings on the Maria Island population only. These data from the Maria Island population thus support Ridpath's predictions that the two males in a group are siblings, at least on Maria Island. Also, the data supports the possibility that a father and son may also make up a breeding group since they are first degree relatives as are siblings and would have similar band sharing coefficients. In general, it is not clear why males remain in polyandrous social groups because in 'sharing' a mate their reproductive success appears to be reduced. However, a male Tasmanian native hen could benefit from this type of behaviour in two ways. First, the males within each group are first degree relatives so each male has the opportunity to pass on genes directly by fathering offspring or, indirectly, by helping raise their relatives offspring. This combination of direct and indirect fitness or inclusive fitness (inclusive fitness; Hamilton 1964) may be higher for a male in a polyandrous group than would be his success if he were paired up with a non-related male or attempted to

establish a monogamous relationship and raise offspring (Maynard Smith and Ridpath 1972). Second, the lifetime per-male productivity in groups may be higher than in monogamous pairs. Evaluating these hypothesis will require additional study of these birds to determine the key factors affecting male reproductive success.

There was a large range in BSCs obtained from both the within group male to male comparisons versus the known sibling comparisons. There are two possible explainations for this variation. First, siblings may either be half or full since in a polyandrous mating system both males have the potential of producing an offspring. However, Gibbs et al. (in prep) found that in most cases only one male in a group sired the young. Secondly, extremely low band sharing values, sometimes even below background levels, may be attributed to lost or orphaned wandering chicks that may be adopted into a new family (Goldizen, pers. comm.).

The VNTR data from the Geeveston population suggested that there was more genetic similarity between the males in the group but the degree of relatedness was not at the same level as first degree relatives. However, there is considerable overlap between band sharing coefficients of within group males and siblings. Therefore, it is likely that in some cases in the Geeveston population first degree male relatives remained together in breeding groups. A possible

explaination for the difference in behaviour in the two populations of Tasmanian native hens with respect to first degree male relatives remaining together was that a decline in the Geeveston population occurred at the time samples were being taken (Goldizen et al. 1993). The group structure may have been unstable since related males within each family unit were dying off and thus regrouping with unrelated males.

Experimental error may occur in the interpretation of the DNA profiles. One problem arises when assigning a particular molecular weight to a band on an autoradiograph due to electrophoretic variation (Galbraith et al. 1991). Each lane could infer a different size band when in fact it is the same band. This problem can be overcome by using an in-lane marker but it still cannot provide 100% accuracy during scoring. Another problem arises when two bands of the same molecular size will overlap each other on the autoradiograph resulting in a darker band. Whether this represents a homozygote at that allele or two separate bands remains unclear. I assumed the darker band to represent a homozygote. A second problem arises when there is variation in the concentration of the DNA samples used to generate the profile. A DNA sample in low concentrations will not generate a signal for light bands, therefore most of the scoring was limited to fairly dark bands. Errors that arise through this technique will not likely effect any conclusions drawn from this study

because of the large number of pair-wise comparisons that were made between individuals. Such errors may have greater importance in studies involving parentage analysis where it is important to trace every band in an offspring back to either parent (e.g. Burke and Burford 1987; Wetton et al. 1987; Burke et al. 1989; Birkhead et al. 1990; Morton et al. 1990; Rabenold et al. 1990; Westneat 1990; Teglestorm et al. 1991).

When using any probe specific for a certain locus of another species, such as VNTR and MHC loci, care should be taken in drawing any conclusions from the data since cross-hybridization techniques may detect other regions which are similar in sequence but not the region of interest. For example, the VNTR probes are specific for human minisatellite 'core' sequence. It is not known if the same corresponding minisatellite regions are the ones viewed in the DNA profiles of Tasmanian native hens. Regardless of the actual location of these loci, inferences can still be made about overall levels of genetic similarity of the populations. The MHC probes on the other hand, were derived from chicken MHC loci. It is possible that the chicken probes may have hybridized to regions of Tasmanian native hen genome which were not, in fact, MHC related. This situation has been observed in other species. For example, Chardon et al. (1985) attributed excessive Class I MHC alleles to pseudogenes in the pig. Others have found a polymorphic MHC-like locus (*Rfp-Y*)

in the chicken genome (Briles et al. 1993). Therefore, when assessing genetic similarity alone, using presumably neutral genetic markers like the VNTR's, it is not important to know the exact function of the alleles in the genome, only that they are a neutral marker. However, when making inferences about the health status of the population as when using the MHC markers, this becomes an important factor.

In addition, complications may arise when applying BSCs as a measurment of genetic similarity in the MHC profiles. Unlike the VNTR profiles which represent many loci, the MHC profiles represent only one locus. Therefore, a series of bands on an individuals profile may be many fragments of one allele. If a group of individuals all shared these bands, a high BSC would result, when in fact only one allele was shared. Overall, MHC profiles may suggest higher levels of genetic similarity between individuals, as was the case for this study, but this should not be a problem when comparisons are made between other BSCs generated from MHC profiles. However, comparisons of BSCs from VNTR and MHC profiles would not be appropriate since the two systems are not on the same relative scale.

Overall, this study has shown how polymorphic loci, the hypervariable minisatellite (Jeffreys 1985b) and the Major Histocompatibility locus, can be used to assess the genetic structure of a bird population. VNTR markers have shown

sufficient variability to allow studies of genetic structure to go beyond between-population comparisons to the level of social groups. Assessing genetic similarity within each mating group has provided information about relationships between individuals which in turn provides information that may help explain the evolution of polyandrous behaviour in these birds. Applying this technique at an inter- and intrapopulation level has shown how versatile this approach can be when looking at conservation biology, population history, possible health status and evolution of behaviour. To further understand the behaviour of the Tasmanian native hen and to extend these findings to the species as a whole, similar genetic analysis should be carried out on other mainland populations. This will address three questions: First, is the lack of genetic variation seen in these birds a reflection of recent past historical events or is, in fact an inherent biological characteristic of this species? Second, are females the dispersing sex in a majority of populations? Finally, do the majority of Tasmanian native hen groups contain adult males that are brothers or fathers and sons which would suggest that the lack of relatedness between males seen in the Geeveston population was a result of anomalous population decline observed in 1989. Research efforts to address these and other questions about the molecular ecology of this species are currently underway.

CHAPTER THREE

HOMOLOGY OF THE CHICKEN MAJOR HISTOCOMPATIBILITY COMPLEX (CLASS I AND II) WITH OTHER AVIAN SPECIES: DETECTION THROUGH CROSS-HYBRIDIZATION

ABSTRACT

The degree of homology and level of variability in DNA-based MHC loci is largly unknown in wild birds. In this chapter, I address these issues in two ways: First, I assessed the degree of MHC sequence homology between the domestic chicken (*Gallus gallus domesticus*) and nine other avian species, by cross-hybridization with inserts from the chicken F10 (Class I MHC) and B-L II (Class II MHC) probes. The degree of homology was established by comparing the MHC profiles, in terms of band intensity and sharpness, between the nine species of birds and the chicken. The B-LβII probe revealed more intense and sharper banding profiles when compared to the F10 probe. Second, I used the B-LβII probe to look at MHC variability in population samples from two bird species, the brown-headed cowbird (*Molothrus ater*) and the common cuckoo (*Cuculus canorus*). *M. ater* revealed higher band sharing coefficients with a mean BSC of 0.94 (SE 0.003) while *C. canorus* individuals showed much greater variability in their banding profiles (mean BSC= 0.23 [SE 0.014]). This study shows how cross-hybridization of the chicken B-LβII probe can be used to assess levels of MHC variability in a wide range of avian species thus eliminating the need to develop species-specific probes. In addition, it

demonstrates that MHC loci can be used to detect variation at the population level.

INTRODUCTION

Many researchers are interested in assessing levels of genetic variability in functional gene systems within wild populations of animals. The MHC (Major Histocompatability Complex) locus is the most variable functional gene family known in the human genome (Klein 1986) and variability of this locus is thought to be controlled by overdominant selection (Takahata and Nei 1990). Until recently, assays of MHC variability has been limited to using serological methods which reveal little about actual levels of variation in MHC loci. However, advances in molecular techniques which have resulted in the creation of DNA probes for particular regions of the MHC loci have now made it possible to directly assess the variability within these regions (e.g. Palmer et al. 1983).

Variability at the MHC locus is thought to be essential in determining an individual's resistance to pathogens. This region of the genome encodes for cell surface glycoproteins which recognize foreign antigens and then present them to T lymphocytes (Klein 1986). This, in turn illicits an immune response to the antigen. Thus, heterozygous MHC loci, have the potential to generate a wide variety of molecules differing at the antigen recognition site

(Buus et al. 1987). When this occurs, the antigen-presenting cell can potentially recognize a larger repertoire of antigens. An increase in homozygosity at this locus may render a population more susceptable to disease. This has been demonstrated in two endangered species, the African cheetah (O'Brien et al. 1985) and the cottontop tamarin (Watkins et al.1990a,b).

One main reason for a decrease in variability in the MHC locus, or at any locus, of naturally occuring populations may be a drastic reduction in population size. Small populations are subject to random genetic drift which, in turn, increases homozygosity. For example, the African cheetah (O'Brien et al 1985), cottontop tamarin (Watkins et al. 1990) and the Tasmanian native hen (Bullough and Gibbs in prep.) all have reduced genetic variability in certain MHC loci as a result of such population bottlenecks.

In this chapter I am interested in determining the homology and levels of variation of the MHC loci of the domestic fowl (*Gallus gallus domesticus*) in other birds. The MHC loci in chickens was first discovered by W. E. Briles in the late 1940s and was named the B complex for its involvement in the blood group system. The B complex is composed of three regions, the B-F (classI), B-L (class II) and B-G (class IV) (Guillemot et al. 1989). Today over twenty species, mostly mammals, two reptiles, an amphibian and the chicken have had

their MHC loci characterized to varying degrees. In this study, I determine the extent to which two chicken B complex probes, F10 (Guillemot et al. 1988) and B-LβII (Bourlet et al. 1988), hybridize to the MHC loci of nine different avian species. My goals are, first to assess the possibility of using the chicken probes to study other MHC loci of other birds without developing species-specific probes for each taxa, and then, second, to assess the amount of variation at the MHC loci at a population level for two species of birds, the brown-headed cowbird (*Molothrus ater*) and and the common cuckoo (*Cuculus canorus*).

METHODS

Samples and Probes: Blood samples were collected by various researchers that have become part of an avian blood collection maintained at McMaster University. The chicken MHC probes B-LβII (0.917 kilobases) and F10 (1.284 kilobases) were received from Charles Auffray, Institut d'Embryologie du CNRS, France. These probes were chosen since these regions of the complex have been shown to be conserved in a wide range of species (Guillemot et al. 1988, 1989)

DNA Extraction: Blood samples were suspended in 3.5 ml of 1X lysis buffer (Seutin et al 1991) and rotated overnight at 37°C. Proteinase K was added to the samples in two, 65 unit doses at 12 hour intervals and rotated at 37°C. DNA was extracted with 70% phenol/30% chloroform twice and once with chloroform, then percipitated with one tenth the volume of 0.3M sodium acetate (pH 5.5) and twice the volume of 95% ethanol. The DNA was then spooled with a hooked glass pipette, washed with 70% ethanol and allowed to air dry for 5 minutes. The DNA was resuspended in approximately 700μl of 1X TNE2 (10 mM Tris-HCl, 10 mM NaCl, 2 mM EDTA) and rotated at 37°C overnight to ensure homogeneity. A TKO 100 Mini-Fluorometer was used to quantify the DNA.

Species Blots: To assess the amount of inter-species homology that was present, I constructed a southern blot on which was included the DNA from two individuals from nine bird species. These species included: mallard duck (*Anas platyrhynchos*), Swainson's thrush (*Catharus ustulatus*), yellow warbler (*Dendroica petechia*), white-throated sparrow (*Zonotrichia albicolis*), brown-headed cowbird (*Molothrus ater*), common cuckoo (*Cuculus canorus*), trumpeter swan (*Cygnus buccinator*), Tasmanian native hen (*Tribonyx morteirii*) and the pukeko (*Porphyrio porphyrio*).   A single sample of chicken (*Gallus gallus*) was used as a control.   In addition, to assess intraspecific variation, I made up two blots, one containing 19 individuals of *M. ater* from Delta Marsh, Manitoba and the other containing an equal number of *C. canorus* individuals from Great Britain.

DNA Digestion and Gel Electrophoresis: *Hin*FI (BMC) was the enzyme chosen for the southern blot analysis since it gave the highest amount of variability with the Tasmanian native hen DNA when labelled with the MHC probes when compared to *Pst*I. *Msp*I and *Tac*I.   Five µg of DNA from each individual on a blot was digested for 5 hours at 37°C with this enzyme.   The cut DNA, loading dye (bromophenol blue), and 1.24 ng of a DNA fingerprint marker cocktail (*Hind*III-, *Eco*RI- and *Bst*EIII- cut lambda DNA) was then run on a 0.8% agarose gel made with

0.5x TBE (0.045M Tris-borate, 0.001M EDTA). To generate the MHC profiles, the DNA was run at 65V for approximately 24 hours in a 20 cm long electrophoresis tank.

Southern Blotting: To prepare the DNA for blotting, the gel was first soaked in a 0.25N HCl solution for 10 minutes, then transfered to a denaturing solution (0.6M NaCl; 0.4M NaOH) for 1 hour, followed by a 1 hour treatment with neutralizing solution (1.5M NaCl; 0.5M Tris; pH 7.2) and then blotted overnight. To do this, the gel was placed on a transfer apparatus (Southern 1975) and the DNA transfered onto a PVDF membrane (Immobilon-N ®). The following day, the membrane was rinsed in 1X SSC and baked at 80°C for 1 hour.

Probing: The MHC blots were prehybridized overnight in a plastic bag with 20ml of a solution consisting of 1.0% SDS, 1.0M NaCl and 10% dextran sulphate and 10 μg/ml of denatured salmon sperm in a 65°C shaking water bath. Inserts of the chicken MHC probes, F10 and B-LβII, were radioactively labelled with 32P dCTP by random primer extention using the Oligolabelling kit from Pharmacia (Feinberg and Vogelstein 1983) and added to the solution and allowed to hybridize overnight at 65°C. Blots were washed (0.1% SDS; 1.0x SSC) two times for 30 minutes at 65°C to remove any background radioactivity. The blots and intensifying screens were placed

in cassettes for 24 hours with Cronex (Dupont) X-ray film and then on Kodak XAR film for 3 to 20 days, depending on the strength of the signal. Before reprobing, blots were stripped with a solution of 0.4M NaOH at 42°C for 45 minutes and then neutralized with 0.5% SDS, 0.1x SSC; 0.2M Tris (pH 7.5) for 45 minutes.

Profile Analysis: To assess cross-species hybridization, the total number of bands and the band sharing coefficients (see below) were generated for each species. The quality of the banding profile in terms of signal strength and sharpness was compared to the chicken profile for all nine species. To rule out the possibility that variation in intensity of signal could be due to differences in DNA concentration, comparisons were made between the same individuals on the same blot hybridized with the two different probes.

To assess the MHC profiles of *M. ater* and *C. canorus,* generated with the probed B-LβII, the bands which were generated through restriction site variation were assigned particular molecular weights and those with the same relative intensity and within 0.5mm of each other were considered to be the same band. The lambda fingerprint cocktail was used as a size reference to ensure that any difference in lane mobility was accounted for when comparing band locations (Galbraith et al. 1991). The total number of bands for each individual was

designated Na or Nb in the comparison and the total number of bands shared between two individuals was designated Nab. The band sharing coefficients (BSCs) were calculated for all pair-wise comparisons of birds using the equation, D = 2Nab/Na+Nb (Lynch 1988). Descriptive statistics were computed using the Minitab program version 5.1.

Experimental error may occur in the interpretation of the MHC profiles. One problem arises when assigning a particular molecular weight to a band on an autoradiograph due to electrophoretic variation (Galbraith et al. 1991). Each lane could infer a different size band when in fact it is the same band. This problem can be overcome by using an in lane marker but it still cannot provide complete accuracy during scoring. Another problem arises when two bands of the same molecular size will overlap each other on the autoradiograph resulting in a darker band. Whether this represents a homozygote at that allele or two separate bands cannot be determined. A third problem arises when there is variation in the concentration of the DNA samples used to generate the profile. A DNA sample in low concentrations will not generate a signal for light bands. For this reason, most of the scoring was limited to fairly dark bands.

RESULTS AND DISCUSSION

Cross-species Hybridization: Figures 3-1 and 3-2 show the results of cross-hybridization experiments using the Class II B-LβII and Class I F10 probes, respectively, for all nine avian species. The Class II B-LβII probe appeared to be more homologous to other avian species when compared to the Class I F10 probe because the banding profiles were more intense and sharper for blots probed with B-LβII. In addition the B-LβII probe gave more scoreable bands in eight out of nine of the species when compared to F10 (Table 3-1). Overall, this suggests that there tends to be fewer *HinF*I restriction sites at the F10 locus and this was consistent with the pattern seen in the chicken. The F10 region also showed less variability when compared to the B-LβII region (Table 3-1) which is consistent with the pattern of higher variation in Class II versus Class I loci seen in other vertebrates (Klein 1986). Conservation of the F10 region is expected since this region recognizes a common property of processed antigen (Guillemot et al. 1988).

This finding of these homology differences is consistent with other studies which show the class II genes to be less divergent both within and between vertebrate species (Bourlet et al. 1988; Guillemot et al. 1989). The B-Lβ gene

in particular is the most highly conserved MHC gene found in mammals and chicken (Bourlet et al. 1988). If significant sequence homology can be maintained in the past 250 million years since the divergence of birds and mammals than it is likely that the more closely related bird species would show high homology at the B-L locus. However, Takahata and Nei (1990), contest the possibility that alleles can remain the same over such a long period of time and propose 30 million years as the maximum time that similar alleles can be maintained. Others, suggest that similar regions in the genes are a result of convergent evolution (Andersson et al. 1991). Regardless of the mechanism which results in similarity of the B-L gene sequences, it still provides support for the idea that this locus is an excellent candidate for cross-hybridization studies of MHC loci in birds.

The F10 region of the chicken Class I gene shows some similarity to the mammalian Class I MHC loci particularly at the α2 domain (Guillemot et al. 1988). However, overall there is reduced similarity between mammals and birds at this locus which is consistent with my results. This lack of similarity is a result of higher divergence rates at the F10 region (Guillemot et al. 1988). The F10 profiles generated in this study did show some homology but to a lesser degree than those created by the B-LβII probes. Initially, the F10 probe appeared to have very little gene homology since the banding

pattern was not sharp and intense. However, closer analysis revealed the chicken profile to also have these same characteristics. This indicated that the F10 probe may have been contaminated which resulted in less intense banding profiles and the presence of background interference. Therefore, the chicken F10 probe may be more homologous to other avian species than my results have shown.

Intraspecific Variation in MHC: To assess intraspecific variation in the two chicken MHC probes, I probed blots consisting of multiple individuals from two species, *C. canorus* and *M. ater,* with both the B-LβII and F10 probes (Fig. 3-3 and 3-4). The quality of the F10 blots of both species was so poor that no reliable information could be obtained from these blots. Generation of reliable F10 results would require further purification of the probe to eliminate any contaminants that may be generating background interference. However, results from the B-LβII probe were clear enough to analyze the two species.

The data showed that each species had quite different levels of variability (Table 3-2). The *M. ater* MHC profiles revealed little polymorphism at this MHC loci, with band sharing values of 0.940 (SE 0.0026) whereas the *C. canorus* individuals had band sharing values of 0.231 (SE 0.014) indicating much higher levels of polymorphism (Table 2-2).

This pattern coincides with what is known about the geographic distribution of samples from each species. The *C. canorus* samples were taken from geographically widespread areas all over Great Britain while the *M. ater* samples were from a single geographic location (Delta Marsh, Manitoba). It is possible that due to effects of philopatry, relatedness between the *M. ater* individuals would be greater, hence the banding profiles would show less genetic divergence. Another possibility for the reduced variation seen in the *M. ater* population is a result of a recent population bottleneck. Limited variation at the MHC class I loci in the African cheetah (*Acinonyx jubatus*) has been attributed to a bottleneck (Yuhki and O'Brien 1990). Also, reduced variation in the MHC profile may be a result of genetic implications of linkage. To eliminate the 'bottleneck' hypothesis, *M. ater* samples should be taken from a wider geographic range and the genetic variability examined at this level. If genetic variability is detected as a result then the geographic hypothesis is the likely explanation for the difference seen in the genetic variability between the two populations. If not then the 'bottleneck' explaination may be the likely explaination.

Care should be taken when interpreting the results of cross-hybridization studies. For example, many MHC class I pseudogenes have been found in the pig during cross-hybridization with human MHC probes (Chardon et al. 1985).

This could very well be the case here, where cross-hybridization techniques are detecting regions of the avian genom which are not functional MHC sequences. In addition, recent studies on the chicken B complex have found a MHC-like gene *Rfp-Y*, which contains similar sequences to both class I and II genes (Briles et al. 1993). Hybridization with class I or II probes could reveal these genes of yet unknown function. Therefore, MHC probes from one species may detect non-functional MHC genes in a different species which may be under completely different set of selection pressures.

In conclusion, this study has shown that the chicken Class II probe B-LβII is a useful probe for cross-hybridization analysis in birds. Assessing the degree of homology at the MHC locus in a wide variety of species through cross-hybridization techniques may reveal evolutionary processes at this locus. The homology that exists at this gene sequence will also allow further research into the population structure of a wide range of bird species thus, providing an alternate route to developeing species-specific probes. Analysis of the genetic structure of *C. canorus* and *M. ater* populations, demonstrate the feasibility of this type of study. In addition, the functional nature of this locus in the immune system may assist conservation biologists in a rapid assessment of the health status of a wide range of endangered bird species.

CHAPTER FOUR: GENERAL CONCLUSIONS

In the preceeding chapters I have shown how the use of DNA-based genetic markers, the hypervariable minisatellite locus (Jeffreys et al. 1985b) and the Major Histocompatibility locus (Klein 1986), have provided information on the population structure and the possible health status of two Tasmanian native hen (*Tribonyx mortierii*) populations. This study is the first to use both types of markers in an analysis of population structure of birds.

At an inter-population level, individuals in both populations had high band sharing coefficients (BSC) indicating that individuals within populations of this species are closely related. However, individuals on Maria Island had BSCs which were on average 10% higher than those in the Geeveston population. This is interpreted to be a result of a unique population bottleneck experienced by this population.

The intra-population genetic analysis of these birds revealed two behavioral characteristics. First, that female hens disperse greater distances than males. Second, a polyandrous trios consist of two first degree male relatives and an unrelated female(s), at least in the Maria Island population. The relationship between the two males was equivelent to that between known siblings in the population.

However, first degree relatives such as brothers and father/son relationships have similar band sharing coefficients, therefore, these two situations could not be distinguished at this point in the study. Further comparisons between known father/son relationships should be carried out to see if these different relationships can be distinguished. As well, behavioural data on the mating groups should resolve this problem.

Two chicken MHC markers were shown to cross-hybridize with nine other avian species. Profile quality varied between species and for different probes. The B-LβII (Class II MHC locus) probe provided the best results when compared to the F10 (Class I MHC locus) probe, suggesting that this region of the avian genome may be more conserved. This sequence homology may allow further analysis of MHC variation in a wide range of birds without developing species-specific probes.

This cross-hybridization technique was expanded to look at population-level variation in these MHC loci in two species of birds, the common cuckoo (*Cuculus canorus*) and the brown-headed cowbird (*Molothrus ater*). Levels of variation, at least at one MHC locus, were considerably higher in the common cuckoo as compared to the brown-headed cowbird. This is interpreted to be a result of differences in the geographical scale over which samples were collected.

In conclusion, the use of DNA markers can broaden our

knowledge of avain population structure, behaviour and relative fitness by looking at genetic variation within and between populations.

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TABLE 2-1. Band sharing coefficients (BSC) for two populations of *T. mortierii* (M=Maria Island, G=Geeveston) compared to values obtained for other vertebrates.

Species	Probe	BSC	Reference
Tribonyx mortierri			This study
(Tasmanian native hen M)	Per	0.68	
(Tasmanian native hen G)	Per	0.58	
(Tasmanian native hen M)	33.6	0.73	
(Tasmanian native hen G)	33.6	0.61	
Equus			Georges et al.
(Horse)	Per	0.27	1988
Canis domesticus			
(Dog)	Per	0.63	
Sus scrofa			
(Pig)[1]	Per	0.68	
Phascolaretos cinereus			Taylor et al.
(Koala)[1]	Per	0.95	1991
(Koala)[1]	33.6	0.97	
Urocyon littoralis			Gilbert et al.
(Channel Island fox)[1]	33.6	0.85	1990
Dipodomys spectabilis			Keane et al.
(Kangaroo rat)	33.6	0.29	1991
Passer domesticus			Burke and
(House sparrow)	33.6	0.28	Burford
Ficedula hypoleuca			1987
(Pied flycatcher)	33.6	0.13	
Miliaria calandra			
(Corn bunting)	33.6	0.12	
Apiaster merops			
(European bee eater)	33.6	0.30	
Lagapus l. scoticus			Hanotte et al.
(Red grouse)	33.6	0.06	1992
Phasianus colchicus			
(Ringneck pheasant)	33.6	0.11	
Coturnix c. japonica			
(Japanese quail)	33.6	0.27	
Gallus gallus			
(Domestic chicken)	33.6	0.29	
Pavo cristatus			
(Indian peafowl)	33.6	0.45	

[1] species which have undergone a recent bottleneck

TABLE 2-2. Band sharing coefficients (BSC) based on MHC profiles obtained from two populations of *T. mortierii* (M=Maria Island, G=Geeveston). The chicken MHC probes, B-LβII and F10, were used to generate the profiles. Similar values for other vertebrate species are included for comparison.

Species		Probe	BSC	Reference
Tribonyx mortierii				
(Tasmanian native hen)	M	B-LβII[1]	0.97	This study
(Tasmanian native hen)	G	B-LβII[1]	0.96	
(Tasmanian native hen)	M	F10	0.93	
(Tasmanian native hen)	G	F10	0.91	
Cuculus canorus				Bullough
(common cuckoo)		B-LβII[1]	0.23	& Gibbs
				(in prep.)
Molothrus ater				
(brown-headed cowbird)		B-LβII[1]	0.94	
Rattus norvegicus				Palmer
(rat)		pH-2IIa	0.58	et al. 1993
Spalax ehrenbergi				Nizetic
(mole rat)		pH-2d-1	0.77	et al. 1985
Sus scrofa				Chardon
(pig)		HLA-B7	0.41	et al. 1985
Mus musculus				Yuhki &
(mouse)		5'Db	0.67	O'Brien
				(1990)
Homo sapiens				
(human)		HLA-B7	0.90	
Felis catus				
(cat)		pFLA 24	0.90	
Acinonyx jubatus				
(African cheetah)		pFLA 24	0.97	
Panthera leo				
(African lion)		pFLA 24	0.93	

[1] indicates a class II MHC probe, all the rest are class I

TABLE 2-3. Average band sharing coefficients (BSC) and standard error of the means (SE) for all pairwise comparisons (N) of unrelated adults for the Maria Island (Maria) and Geeveston (Geeve.) populations of *T. mortierii*. Part A, estimates of genetic similarity through VNTR loci profiles using two multilocus probes, Per and Jeffreys 33.6. Part B, estimates of genetic similarity at the MHC loci, using two chicken B complex probes, B-LβII and F10. Levels of significance (p) were generated through a two-sample rank test, the Wilcoxson Rank Sum Test.

		A. VNTR PROFILES				B. MHC PROFILES			
Probe		PER		JEFFREYS 33.6		B-LβII		F10	
Population		Maria	Geeve	Maria	Geeve	Maria	Geeve	Maria	Geeve
Mean BSC		0.682	0.582	0.728	0.606	0.971	0.962	0.939	0.912
SE		0.007	0.008	0.007	0.007	0.002	0.002	0.003	0.004
N		119	186	118	186	182	169	182	182
Sample Size		25	32	25	32	28	27	28	28
Rank Test	Z	7.69		10.13		-2.66		4.41	
	P	<0.0001		<0.0001		0.0077		<0.0001	

TABLE 2-4. Mean band sharing coefficients (BSC) and standard errors of the mean (SE) for all pairwise comparisons (N) between members of the same sex (M/M=male to male, F/F=female to female) for each population of *T. mortierii*. VNTR profiles revealed estimates of genetic similarity using two multilocus probes, Per and Jeffreys 33.6. Levels of significance (p) were generated through a rank sum (Wilcoxson Rank Sum) test.

		MARIA ISLAND POPULATION				GEEVESTON POPULATION			
Probe		PER		JEFFREYS 33.6		PER		JEFFREYS 33.6	
Sex Group		M/M	F/F	M/M	F/F	M/M	F/F	M/M	F/F
Mean BSC		0.681	0.665	0.760	0.689	0.604	0.560	0.615	0.603
SE		0.012	0.021	0.011	0.017	0.019	0.015	0.013	0.017
N		43	19	42	18	40	47	42	46
Sample Size		15	10	15	10	15	16	15	16
Rank test	z	- 0.81		- 3.25		2.05		0.92	
	p	0.4177		0.0012		0.0408		0.3597	

TABLE 2-5 Mean band sharing coefficients (BSC) and standard errors of the mean (SE) for all intragroup comparisons (N) of adult *T. mortierii* within polyandrous groups at Maria Island. Comparisons include BSC from the following sets of adults: male to female (M/F); male to male (M/M); and female to female (F/F). Two-sample rank test (Wilcoxson Rank Sum) tested level of significance (p) for each comparison.

		MARIA ISLAND POPULATION					
Probe		PER			JEFFREYS 33.6		
Comparison		M/F	M/M	F/F	M/F	M/M	F/F
Mean BSC		0.680	0.804	0.695	0.777	0.847	0.810
SE		0.016	0.016	0.045	0.017	0.026	0.040
N		16	7	2	17	7	2
Sample Size		23	10	4	23	11	4
Rank test M/F M/M	z	2.81			2.11		
	p	0.0049			0.0352		
Rank test M/M F/F	z		− 1.92			− 0.732	
	p		0.0550			0.4642	

TABLE 2-6 Mean band sharing coefficients (BSC) and standard errors of the mean (SE) for all intragroup comparisons (N) of adult *T. mortierii* from polyandrous groups at Geeveston. Comparisons include BSC from the following sets of adults: male to female (M/F); male to male (M/M); and female to female (F/F). Genetic data is from two multilocus probes, Per and Jeffreys 33.6. Two-sample rank test (Wilcoxson Rank Sum) was used to test the level of significance (p) for each comparison.

			GEEVESTON POPULATION				
Probe			PER			JEFFREYS 33.6		
Sets of Adults		M/F	M/M	F/F	M/F	M/M	F/F
Mean BSC		0.643	0.698	0.560	0.629	0.690	0.581
SE		0.018	0.059	0.031	0.014	0.036	0.039
N		30	9	8	29	9	9
Sample Size		31	10	12	31	10	12
Rank test of M/F M/M	z	1.67			1.96		
	p	0.0953			0.0499		
Rank test of M/M F/F	z		-1.88			1.81	
	p		0.0603			0.0700	

TABLE 2-7. Mean band sharing coefficients (BSC) and standard errors of the mean (SE) for one intrapopulation and two intragroup comparisons of the *T. mortierii* population on Maria Island. Comparisons include BSC for the following sets of individuals: males within the population (MP); males within a group (MG); and siblings within a group (Sib). Genetic data is from two multilocus probes, Per and Jeffreys 33.6. Two-sample rank test (Wilcoxson Rank Sum) was used to test the level of significance (p).

			MARIA ISLAND POPULATION					
Probe			PER			JEFFREYS 33.6		
Individuals			MP	MG	SIB	MP	MG	SIB
Mean BSC			0.681	0.804	0.802	0.760	0.847	0.841
SE			0.012	0.016	0.010	0.011	0.026	0.013
N			43	7	56	42	7	56
Sample Size			15	10	28	15	11	28
Rank test of M/F M/M	z		3.14			2.53		
	p		0.0006			0.0113		
Rank test of M/M F/F	z			-0.10			-0.09	
	p			0.9210			0.9305	

TABLE 2-8 Mean band sharing coefficients (BSC) and standard errors of the mean (SE) for one intrapopulation and two intragroup comparisons of the *T. mortierii* population at Geeveston. Comparisons include BSC from the following sets of individuals: males within the population (MP); males within a group (MG); and siblings within a group (F/F). Genetic data is from two multilocus probes, Per and Jeffreys 33.6. Two-sample rank test (Wilcoxson Rank Sum) was used to test level of significance (p).

			GEEVESTON POPULATION					
Probe			PER			JEFFREYS 33.6		
Individuals			MP	MG	SIB	MP	MG	SIB
Mean BSC			0.604	0.698	0.738	0.615	0.690	0.833
SE			0.019	0.059	0.028	0.013	0.036	0.028
N			40	9	12	42	9	12
Sample Size			15	10	13	15	10	13
Rank test of M/F M/M	z		2.00			2.18		
	p		0.0452			0.0294		
Rank test of M/M F/F	z			-0.11			-2.96	
	p			0.92			0.0031	

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TABLE 3-1. The mean number of bands per species (n), band
sharing coefficients (BSC) between the two
individuals of each species on the multi-species
blot and quality of banding profile (Q) compared
to the chicken profile (S=strong signal; W=weak
signal), using two chicken MHC probes, the class
I F10 and class II B-LβII, on nine avian species.

| | | Probes | | | | |
| | B-Lβ II | | | F10 | | |
Species	n	BSC	Q	n	BSC	Q
Gallus gallus (domestic chicken)	21	–	–	13	–	–
Cuculus conorus (common cuckoo)	20	0.41	W	7	0.62	W
Molothrus ater (brown-headed cowbird)	23	0.58	S	16	1.00	W
Tribonyx mortierii (Tasmanian natve hen)	23	0.93	W	14	1.00	W
Anas platyrhynchos (mallard duck)	22	0.65	S	13	0.48	S
Catharus ustulatus (Swainson's thrush)	25	0.45	S	14	0.96	W
Dendroica petechia (yellow warbler)	21	0.67	W	5	0.80	W
Zonotrichia albicolis (white throated sparrow)	23	0.62	S	14	1.00	W
Cygnus buccinator (Trumpeter swan)	14	0.86	W	4	1.00	W
Pophyrio pophyrio (pukeko)	24	0.68	W	9	0.78	S

TABLE 3-2. Descriptive statistics for the mean band sharing coefficients (BSC) for all possible pair-wise (N) comparisons between a) *C. canorus* individuals and b) *M. ater* individuals. Both profiles were hybridized with the chicken class II B-LβII probe. Nineteen individuals (n) of each species were compared.

			Descriptive Statistics			
	N	n	Mean BSC	SE	Min BSC	Max BSC
a) Common cuckoo (*Cuculus canorus*)	171	19	0.2305	0.0140	0.00	0.89
b) Brown-headed cowbird (*Molothrus ater*)	171	19	0.9404	0.0026	0.86	1.00

FIGURE 2-1   Map of Tasmania, Australia, showing the locations of the two populations of Tasmanian native hens (*Tribonyx mortierii*) understudy.

AUSTRALIA

TASMANIA

TASMANIA

GEEVESTON
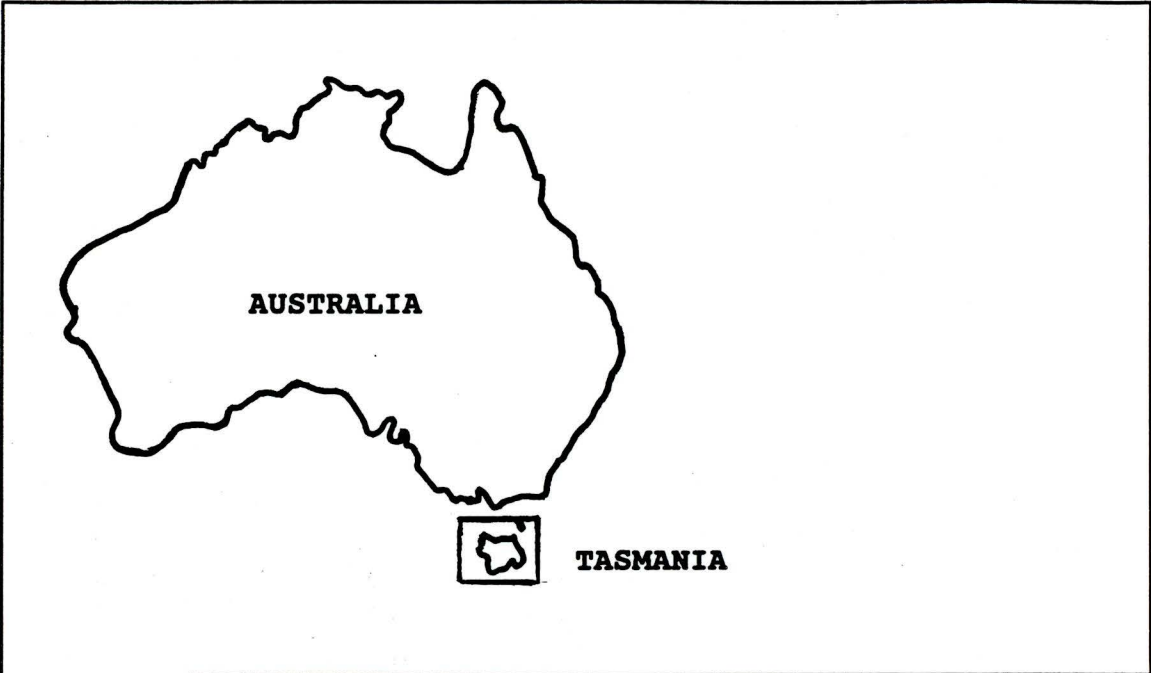
MARIA ISLAND

FIGURE 2-2   Hypervariable minisatellite DNA profiles
               generated using the probe Per from fourteen
               adult Tasmanian native hens (*Tribonyx
               mortierii*) on Maria Island. The identification
               number, gender (M=male, F=female) and relative
               molecular weight (kb=kilobase) of the bands are
               provided for each individual.
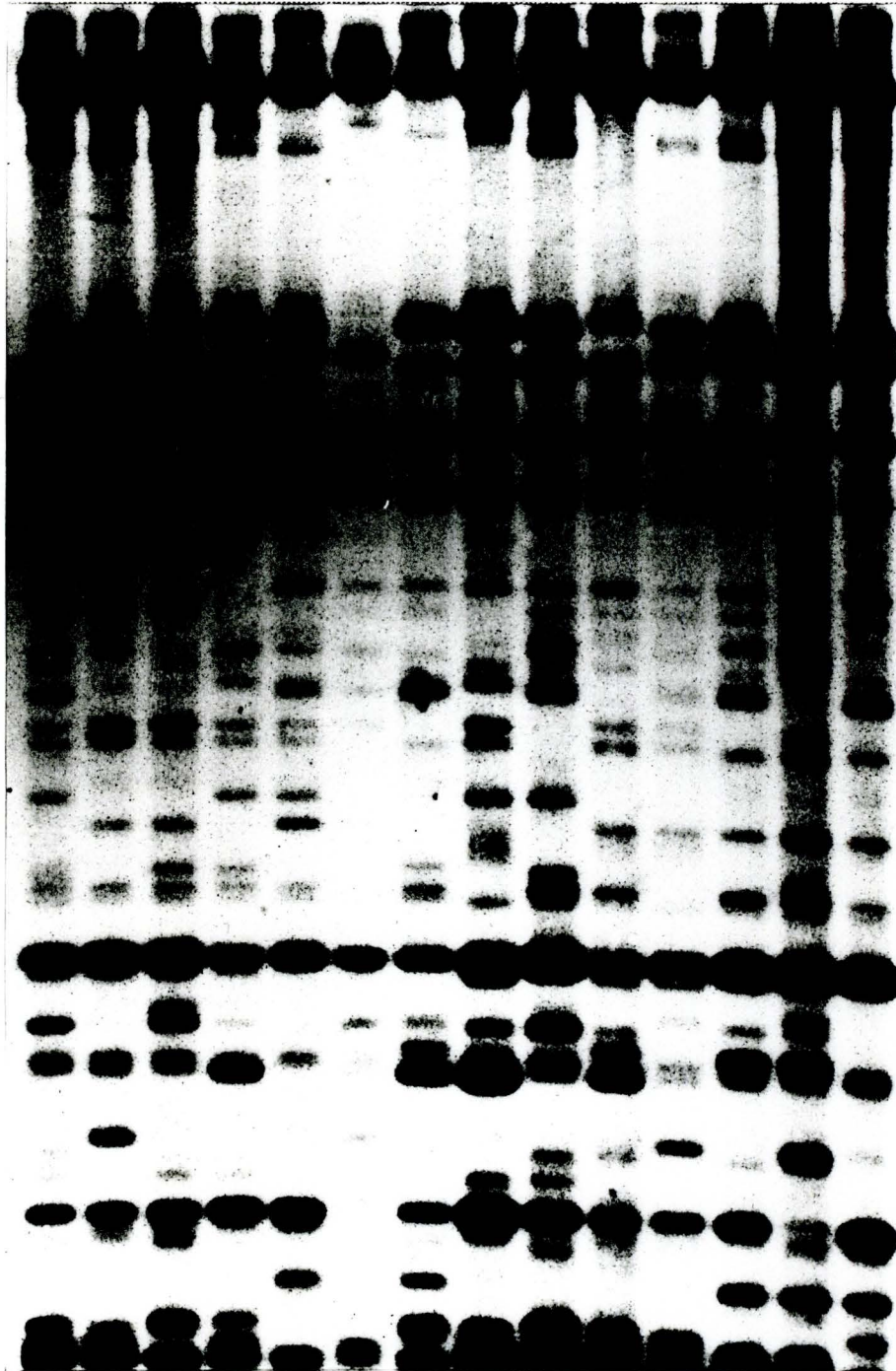
FIGURE 2-3   Hypervariable minisatellite DNA profiles
generated using the probe Per from sixteen
adult Tasmanian native hens (*Tribonyx
mortierii*) from Geeveston. The identification
number, gender (M=male, F=female) and relative
molecular weight (kb=kilobase) of the bands are
provided for each individual.

M F F M M F F M M M F F M F M F

43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 kb

20

5.0

2.5
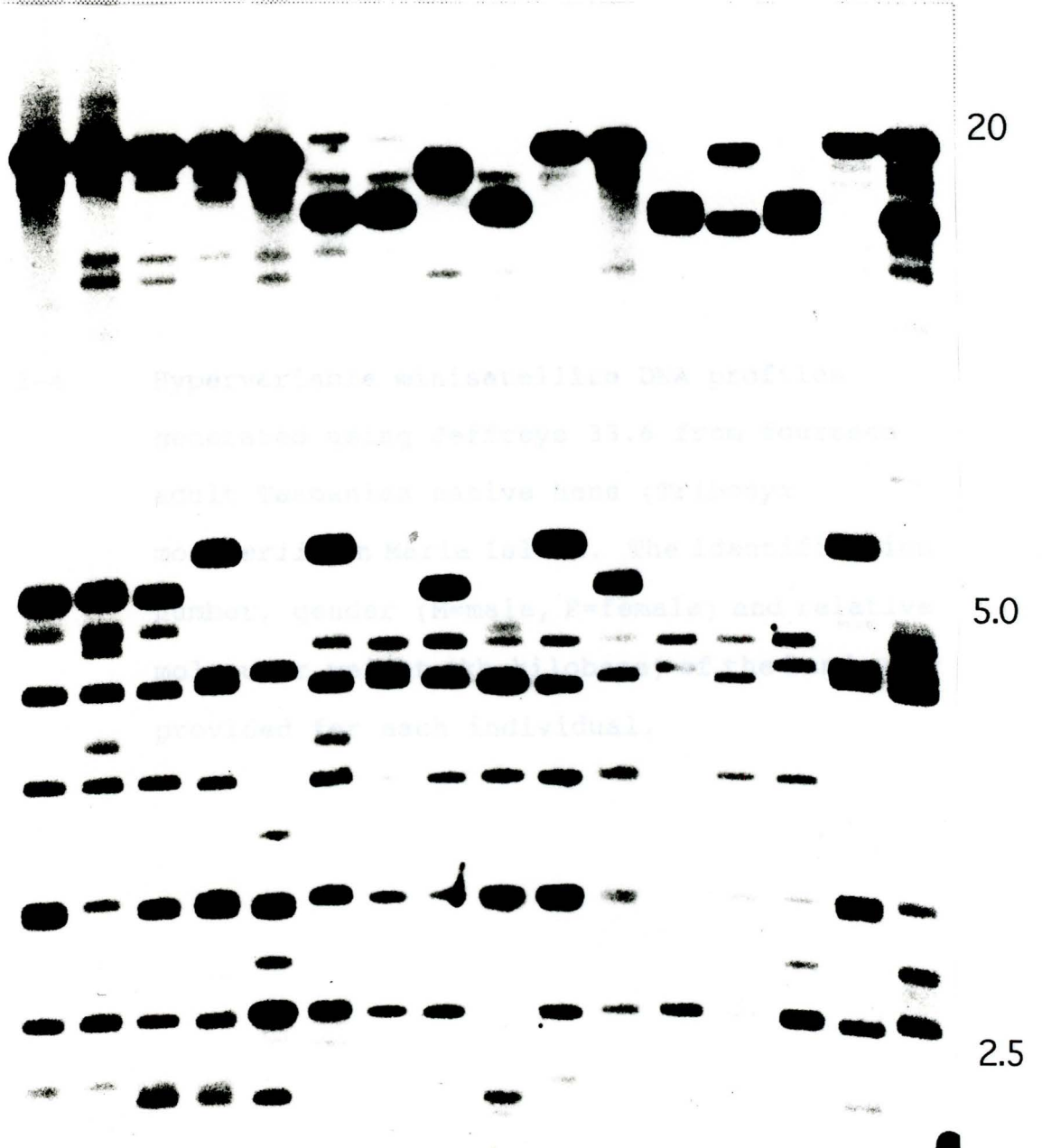
FIGURE 2-4   Hypervariable minisatellite DNA profiles
              generated using Jeffreys 33.6 from fourteen
              adult Tasmanian native hens (*Tribonyx*
              mortierii) on Maria Island. The identification
              number, gender (M=male, F=female) and relative
              molecular weight (kb=kilobase) of the bands are
              provided for each individual.

M M F M   F M M M F F M F M
14 15 16 17 _ 18 19 20 21 22 23 24 25 26   kb
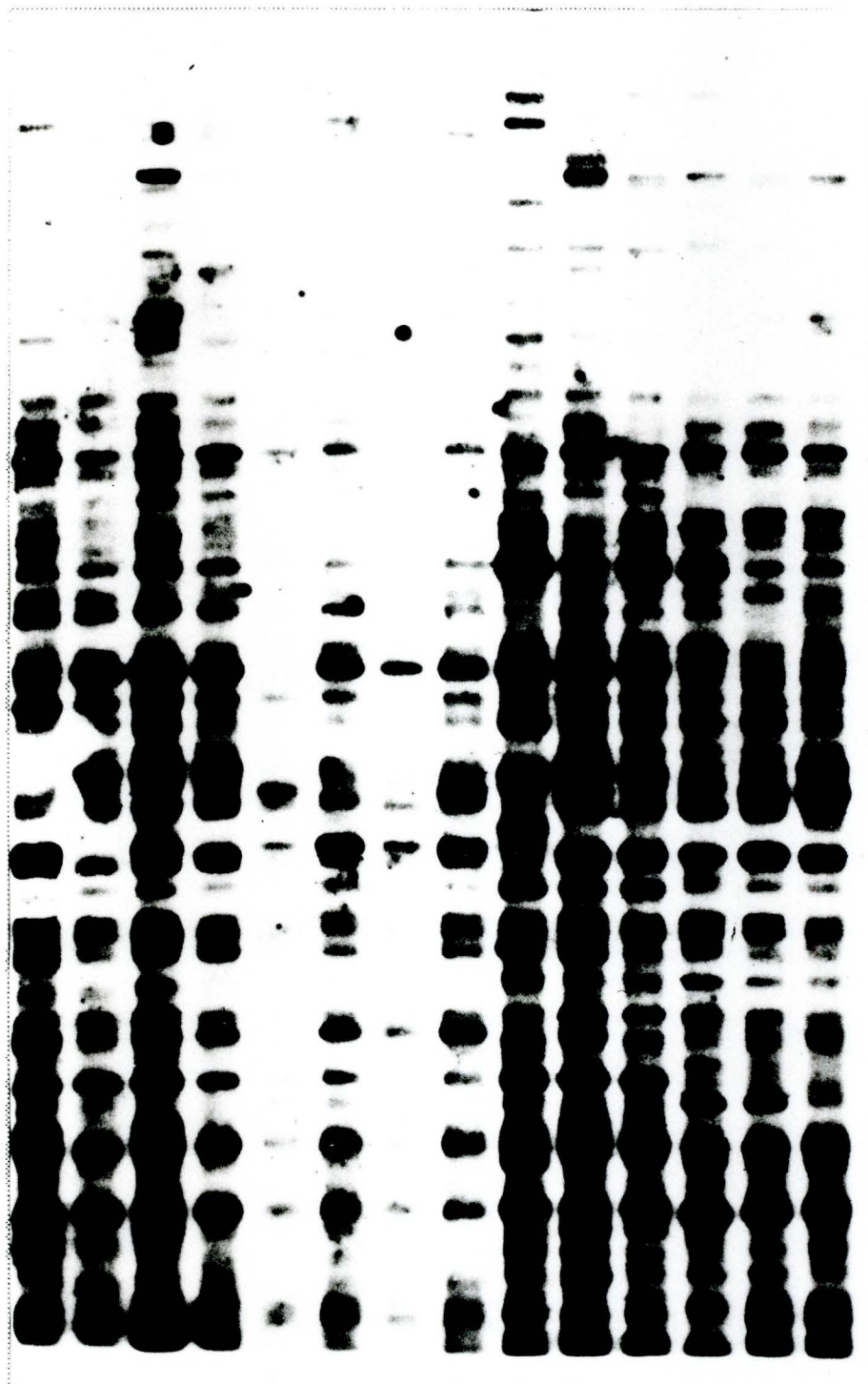
20

5.0

2.5

FIGURE 2-5   Hypervariable minisatellite DNA profiles
generated using Jeffreys 33.6 from sixteen
adult Tasmanian native hens (*Tribonyx
mortierii*) from Geeveston. The identification
number, gender (M=male, F=female) and relative
molecular weight (kb=kilobase) of the bands are
provided for each individual.
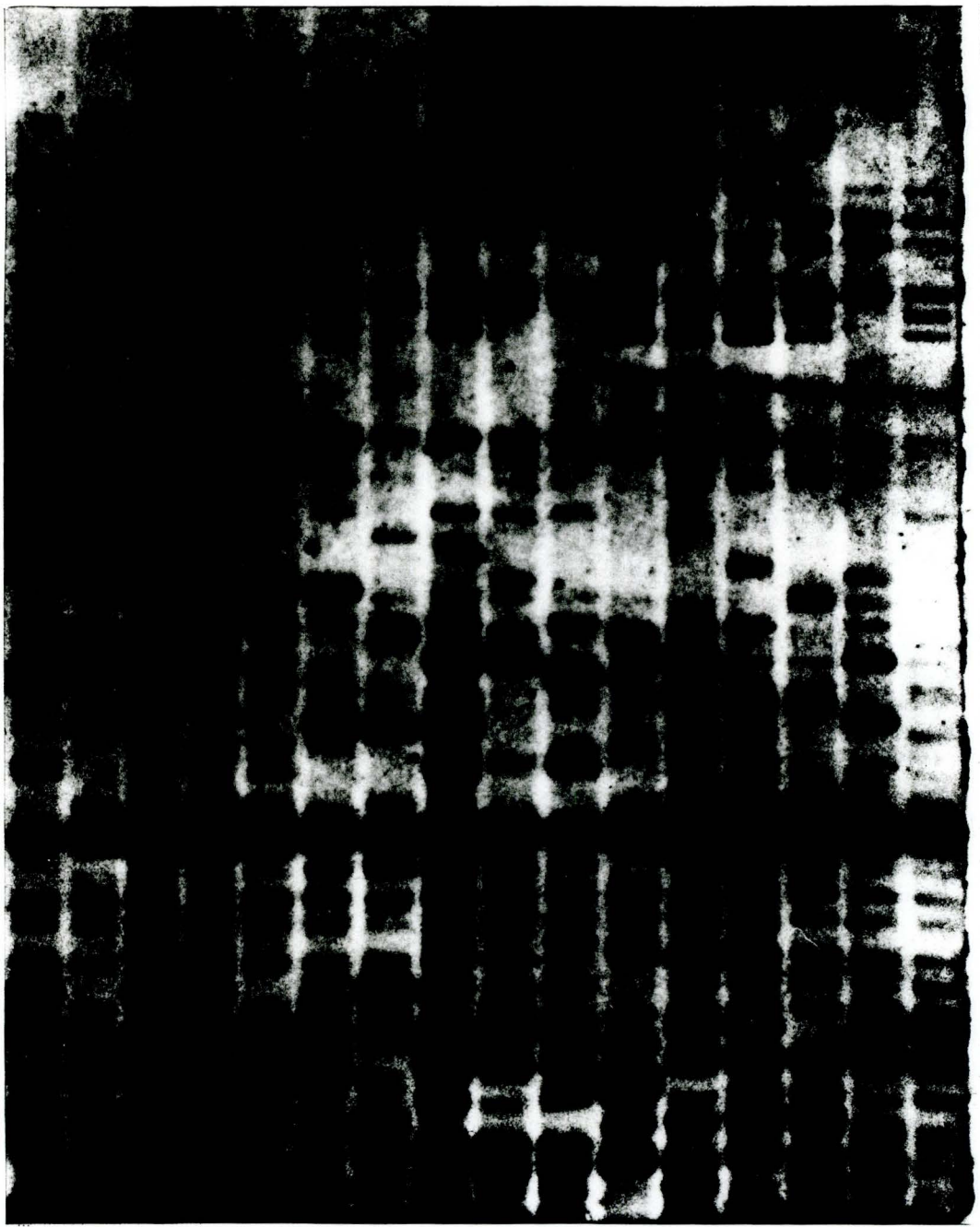
FIGURE 2-6   Major Histocompatibility DNA profiles generated using the B-LβII probe from fourteen adult Tasmanian native hens (*Tribonyx mortierii*) on Maria Island. The identification number, gender (M=male, F=female) and relative molecular weight (kb=kilobase) of the bands are provided for each individual.

M M F M F M F M M F F F M M kb
1 2 3 4 5 6 7 8 9 10 11 12 13 14

20

5.0

2.0
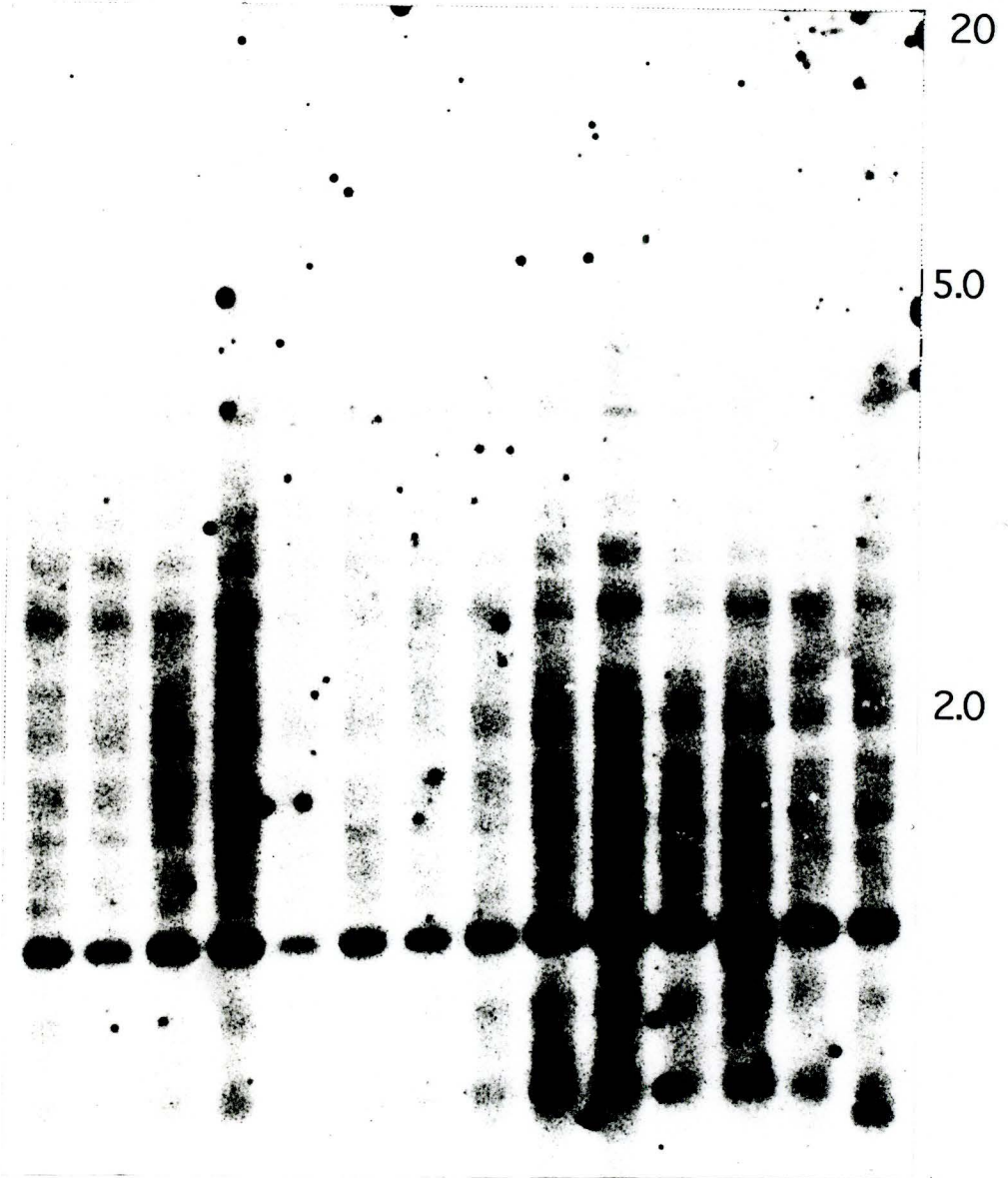
FIGURE 2-7   Major Histocompatibility DNA profiles generayed using the B-LβII probe from fourteen adult Tasmanian native hens (*Tribonyx mortierii*) from Geeveston. The identification number, gender (M=male, F=female) and relative molecular weight (kb=kilobase) of the bands are provided for each individual.

M	F	F	M	M	F	F	M	M	M	F	F	M	F	kb
43	44	45	46	47	48	49	50	51	52	53	54	55	56	

20

5.0

2.0
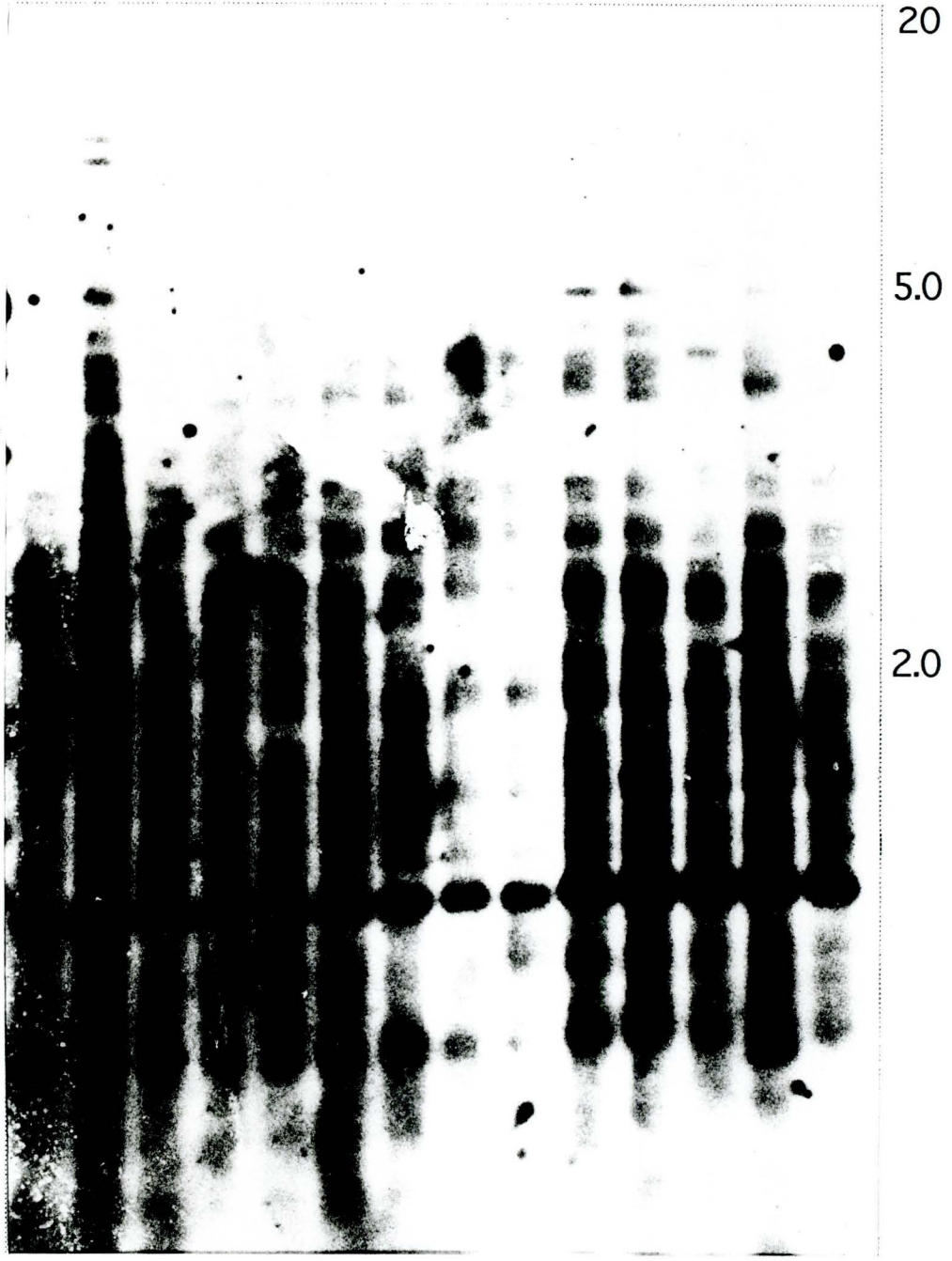
FIGURE 2-8   Major Histocompatibility DNA profiles generated
              using the F10 probe from fourteen adult
              Tasmanian native hens (*Tribonyx mortierii*) on
              Maria Island. The identification number,
              gender (M=male, F=female) and relative
              molecular weight (kb=kilobase) of the bands are
              provided for each individual.
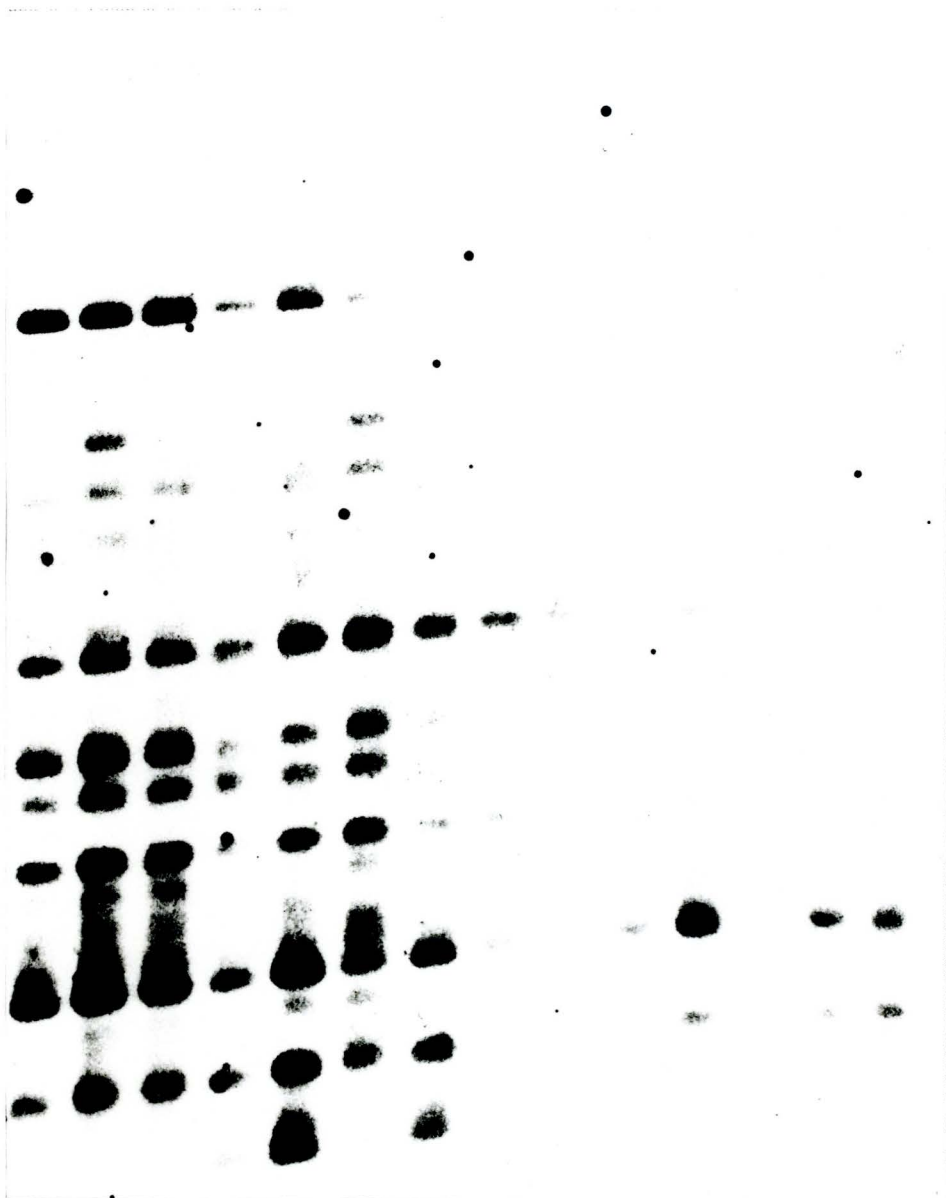
FIGURE 2-9   Major Histocompatibility DNA profiles generated using the F10 probe from fourteen adult Tasmanian native hens (*Tribonyx mortierii*) from Geeveston. The identification number, gender (M=male, F=female) and relative molecular weight (kb=kilobase) of the bands are provided for each individual.

FIGURE 3-1   Major Histocompatibility DNA profiles from
ten avian species generated using the B-LβII
probe. From left to right, the domestic
chicken (D), pukeko (P), Tasmanian native hen
(N), brown headed cowbird (B), common cuckoo
(C), trumpeter swan (T), white-throated sparrow
(W), yellow warbler (Y), Swainson's thrush (S)
and the mallard duck (M). The relative
molecular weight (kb=kilobase) of the bands are
provided for each individual.

D P P N N B B C C T T W W Y Y S S M M   kb

2.0

5.0
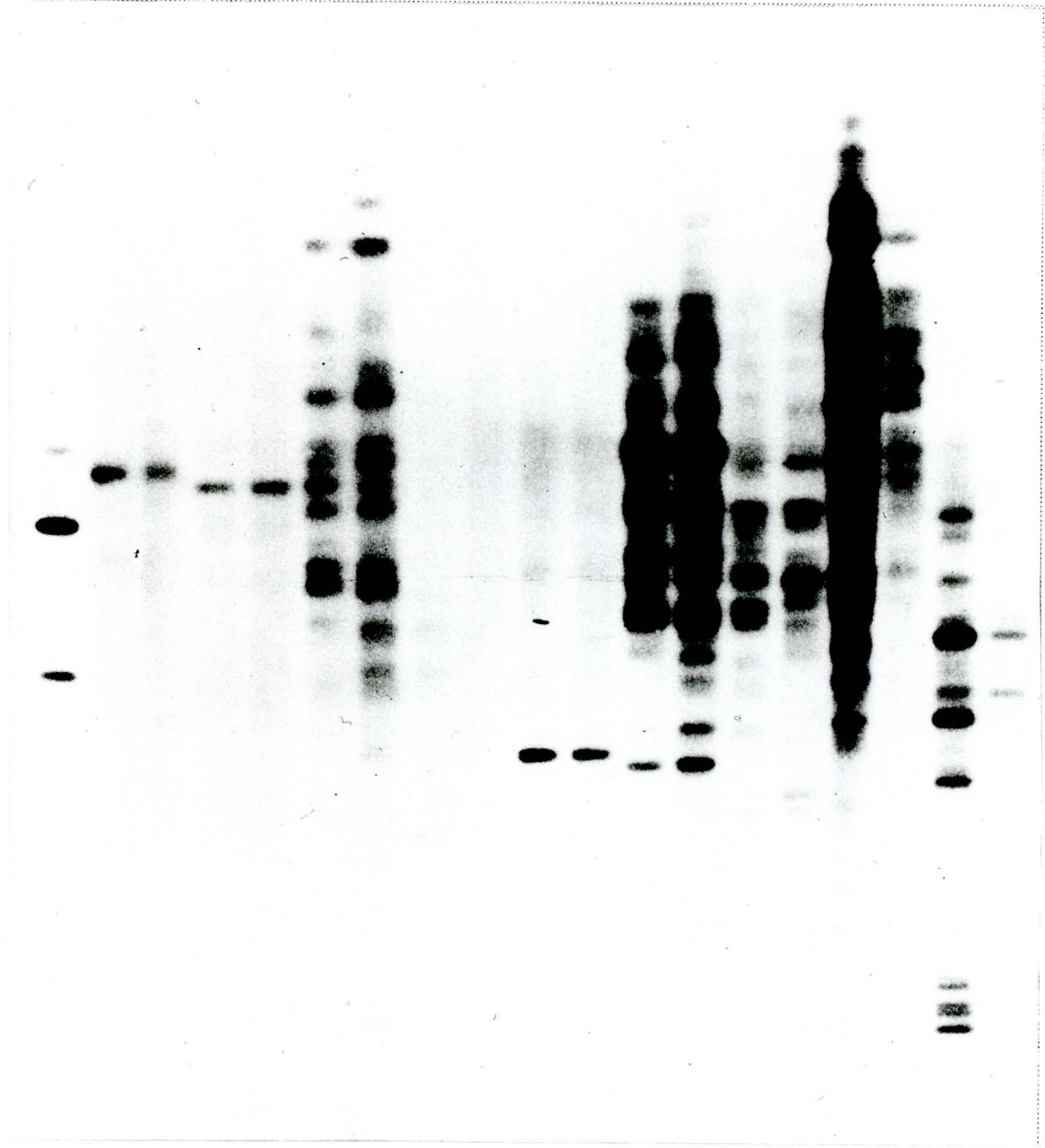
FIGURE 3-2   Major Histocompatibility DNA profiles generated using the F10 probe from ten avian species. From left to right, the domestic chicken (D), pukeko (P), Tasmanian native hen (N), brown headed cowbird (B), common cuckoo (C), trumpeter swan (T), white-throated sparrow (W), yellow warbler (Y), Swainson's thrush (S) and the mallard duck (M). The relative molecular weight (kb=kilobase) of the bands are provided for each individual.

D P P N N B B C C T T W W Y Y S S M M

kb
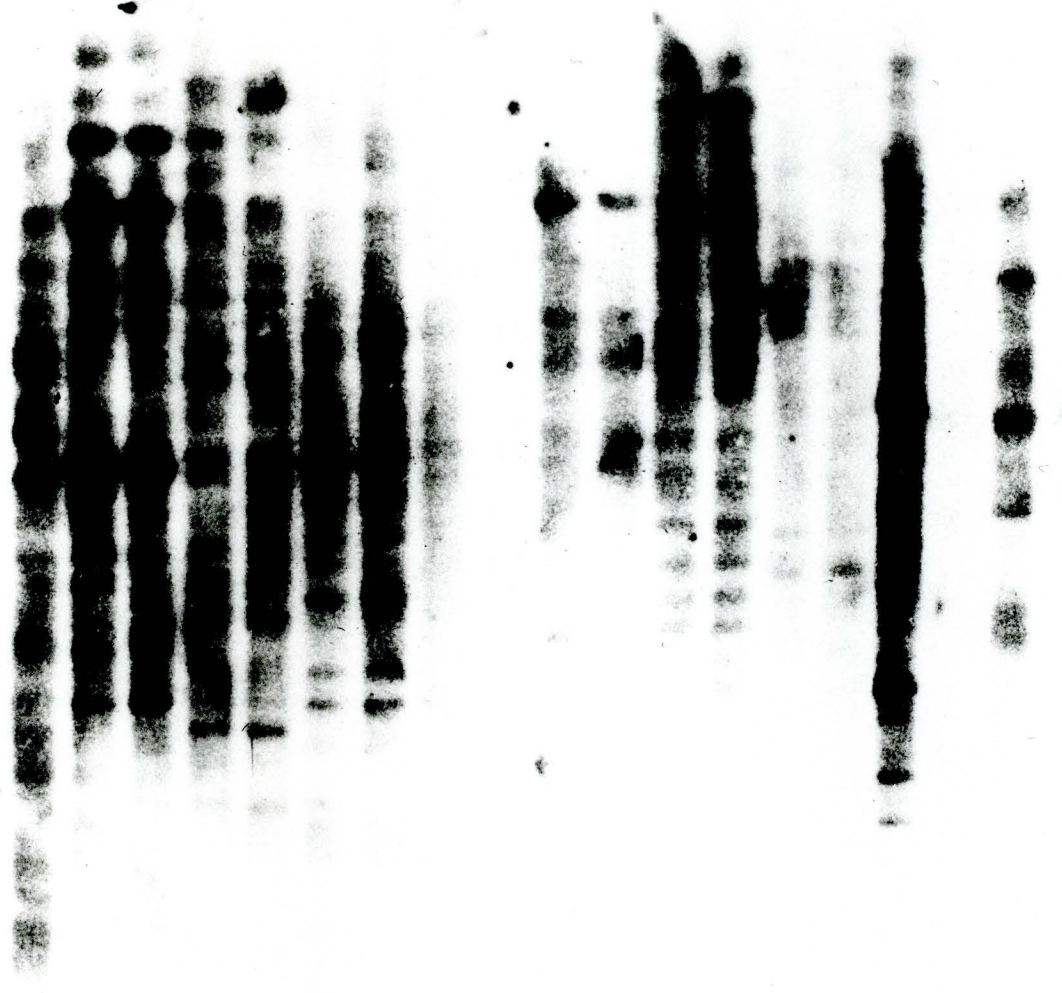
2.0

5.0

FIGURE 3-3   Major Histocompatibility DNA profiles generated using the B-LβII probe from nineteen adult common cuckoos (*Cuculus canorus*) from Great Britain.   The identification number and relative molecular weight (kb=kilobase) of the bands are provided for each individual.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

kb

5.0

2.0
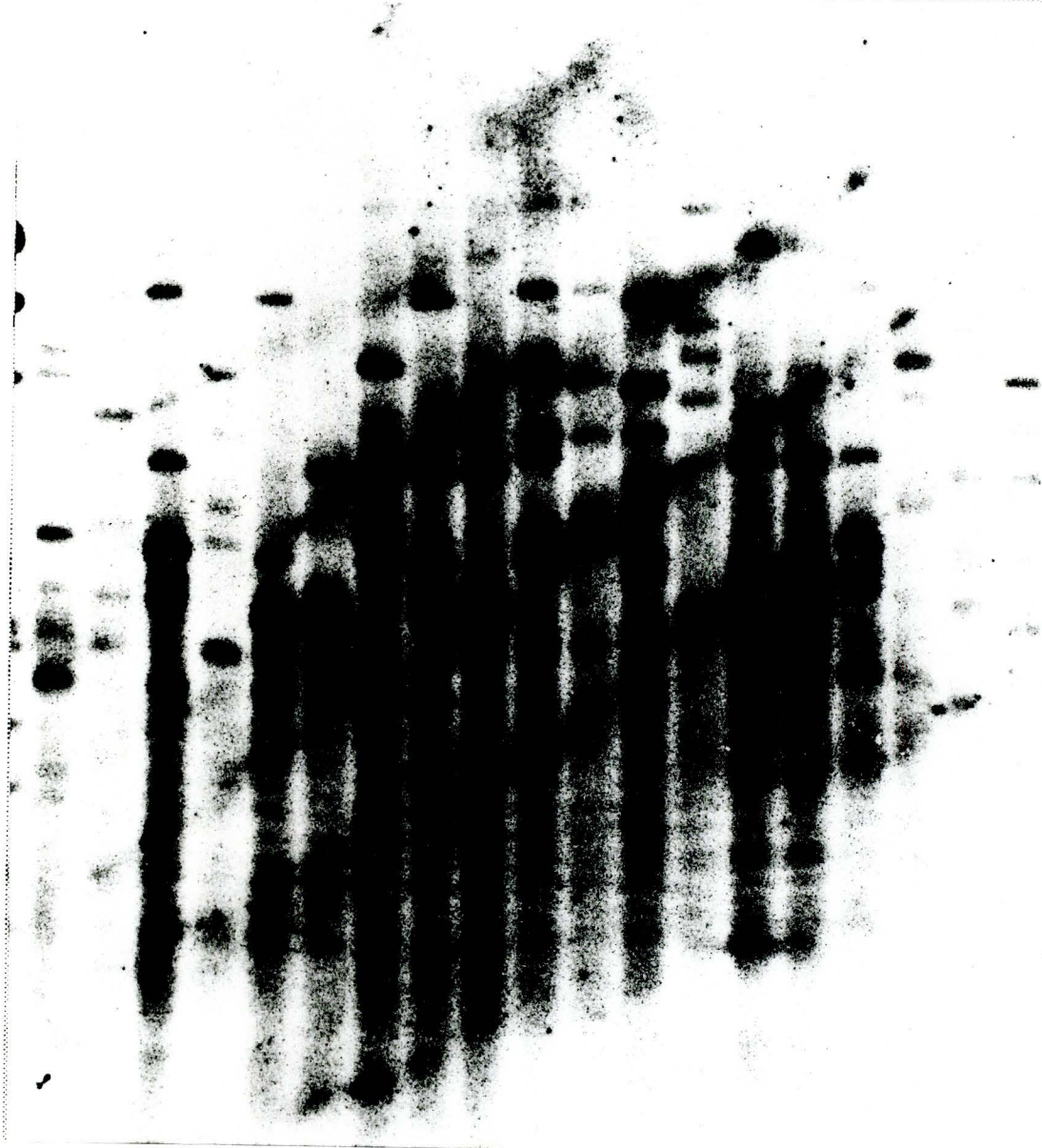
FIGURE 3-4   Major Histocompatibility DNA profiles generated using the B-LβII probe from nineteen adult brown-headed cowbirds (*Molothrus ater*) from Delta Marsh, Manitoba. The identification number and relative molecular weight (kb=kilobase) of the bands are provided for each individual.
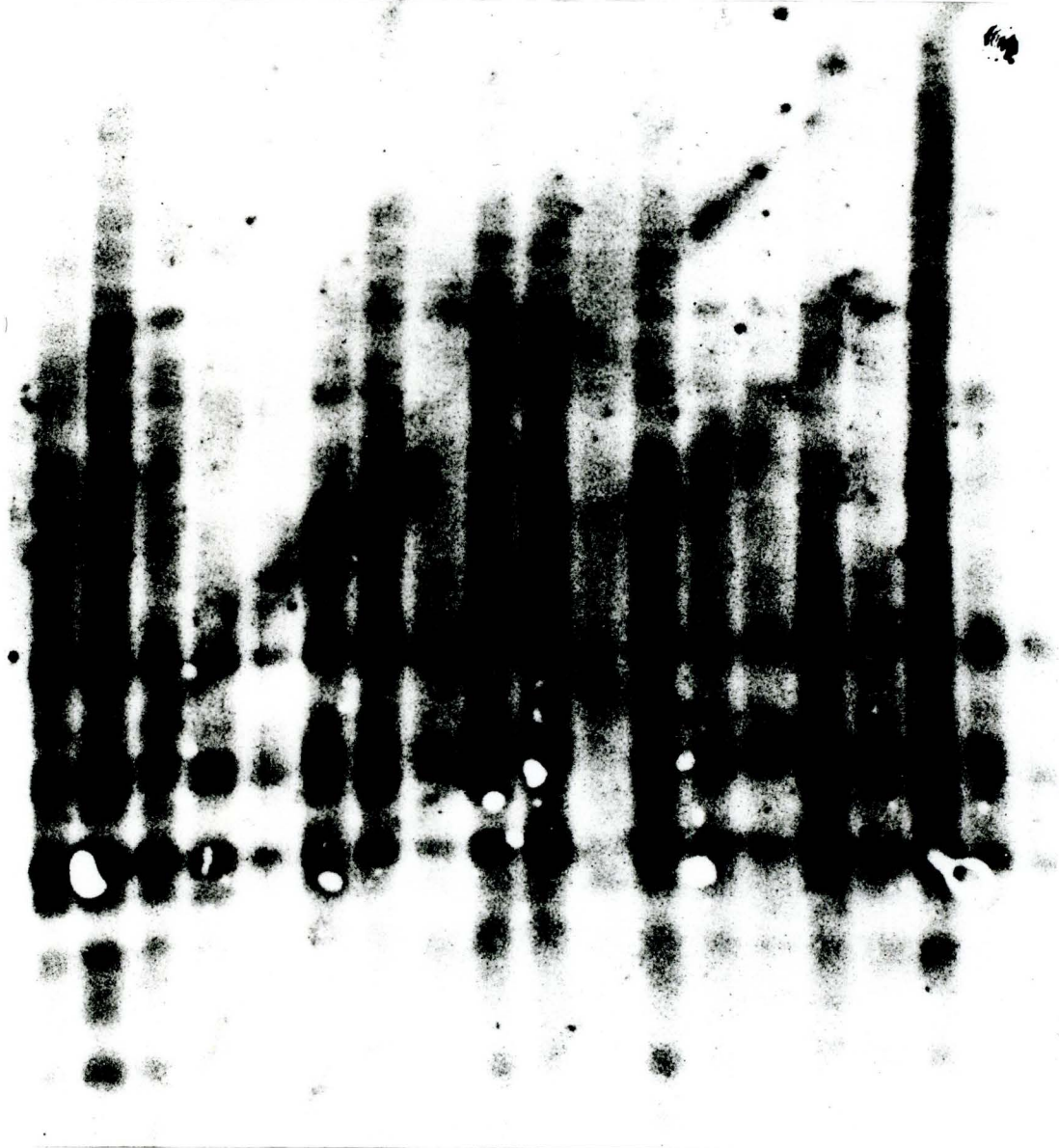
APPENDIX 2-1.   Band sharing coefficients (x100) for two VNTR loci calculated from all pairwise comparisons between adult *T. mortierii* on Maria Island. Numbers on the outside of the table identify individual birds. Values above the diagonal are based on blots probed with Per while those below the diagonal are based on blots probed with Jeffreys 33.6.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1		61	80	82	74	68	73	63	73	52	59	73	62
2	73		85	56	63	69	63	64	67	68	75	73	67
3	93	76		70	68	77	61	79	71	62	66	71	68
4					61	60	68	62	68	55	69	86	64
5	68	74	58			68	69	70	70	62	74	69	73
6	68	57	67		61		71	72	61	76	72	61	81
7	77	78	79		73	78		60	62	81	67	69	76
8	65	71	63		67	62	67		56	68	81	67	67
9	79	71	82		67	58	75	73		67	63	76	66
10	70	57	63		57	86	80	63	73		64	70	80
11	59	65	58		65	78	78	57	71	76		74	77
12	71	68	70		64	64	68	65	83	65	77		76
13	72	69	79		65	86	88	67	79	76	73	68	

APPENDIX 2-1 continued.

	14	15	16	17	18	19	20	21	22	23	24	25	26
14		76	59	57	56	83	56	61		70	68	59	59
15	92		68	67	65	68	60	65		82	76	68	78
16	78	79		72	63	61	63	69		61	65	56	67
17	76	73	83		76	65	76	72		75	78	59	76
18	84	81	76	79		51	76	65		73	76	73	63
19	71	68	74	85	76		56	63		72	70	48	73
20	73	72	60	67	76	67		82		73	72	91	68
21	84	70	77	78	79	80	88			84	82	74	74
22	78	72	67	69	71	72	86	88					
23	78	75	67	74	82	72	82	85	74		88	77	82
24	80	75	69	75	87	77	79	89	82	86		75	80
25	76	73	64	73	81	70	89	86	81	93	85		61
26	73	72	71	74	73	74	76	81	82	78	80	74	

APPENDIX 2-2. Band sharing coeifficients (x100) for two VNTR loci calculated from all
pairwise comparisons between adult *T. mortierii* at Geeveston. Numbers
on the outside of the table identify individual birds. Values above the
diagonal are based on blots probed with Per while those below the
diagonal are based on blots probed with Jeffreys 33.6.

	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
27		83	69	69	67	55	68	74	76	51	65	71	56	55	76	67
28	74		86	76	86	74	79	86	82	59	73	72	59	57	77	67
29	85	74		73	82	71	65	72	73	50	69	74	61	65	67	70
30	71	57	68		84	52	71	66	74	49	69	74	61	65	67	70
31	73	71	71	78		63	52	76	78	47	67	67	65	50	57	75
32	55	55	59	45	44		57	70	72	70	47	61	52	50	50	44
33	62	68	60	47	50	56		81	82	54	67	61	65	51	58	69
34	65	72	60	55	62	52	69		79	61	63	63	54	41	53	65
35	63	77	63	47	58	56	75	68		63	50	70	69	50	56	67
36	54	58	46	36	40	62	60	55	63		50	51	36	47	47	65
37	68	68	71	55	66	53	62	62	64	48		58	62	40	77	67
38	69	62	73	63	63	65	63	55	69	56	66		56	49	68	67
38	62	62	54	48	52	57	63	55	55	61	48	64		58	52	58
40	60	57	64	51	55	56	50	54	57	55	51	55	59		50	63
41	73	50	65	44	56	90	51	55	47	52	78	64	52	47		64
42	56	60	56	58	62	47	69	61	60	64	62	58	54	57	50	

APPENDIX 2-2 continued.

	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
43		71	76	81	38	41	50	58	52	55	69	26	47	45	57	34
44	74		79	56	44	47	61	70	57	55	67	57	53	57	67	56
45	76	81		78	56	47	50	65	63	48	61	46	53	46	82	51
46	61	73	70		59	50	35	44	48	52	52	45	44	42	81	49
47	62	77	69	70		44	47	44	48	52	39	61	63	42	65	70
48	67	80	74	70	79		72	59	57	61	55	57	65	46	51	46
49	60	62	68	68	59	63		63	48	58	65	79	69	67	54	54
50	41	50	51	49	60	55	43		58	62	83	52	67	45	46	46
51	56	45	60	43	56	70	48	55		53	53	56	52	50	56	44
52	67	72	69	65	74	79	64	51	65		64	47	55	40	47	53
53	65	67	68	58	68	68	57	68	54	73		47	62	47	47	53
54	56	54	60	47	65	65	58	54	76	60	47		65	75	56	72
55	47	51	57	61	67	67	55	62	51	52	60	63		58	46	57
56	57	55	61	55	65	70	64	51	79	74	55	75	67		44	61
57	70	67	73	79	68	63	62	49	59	68	52	63	55	64		60
58	52	72	74	75	70	70	60	55	57	65	59	60	71	70	73	

APPENDIX 2-3. Band sharing coefficients (BSC) for all pair-
wise comparisons between *T. mortierii* siblings
using the multilocus DNA probes, Per and
Jeffreys 33.6. Values in part A were generated
from the Geeveston population and part B from
the Maria Island population.

	Population	Probe	BSC			
A.	Geeveston	Per	0.78	0.64	0.88	0.85
			0.77	0.71	0.63	0.63
			0.75	0.62	0.71	0.89
		33.6	0.87	0.87	0.87	0.89
			0.93	0.86	0.83	0.88
			0.81	0.54	0.81	0.83
B.	Maria Island	Per	0.77	0.79	0.76	0.80
			0.79	0.75	0.95	0.88
			0.63	0.68	0.76	0.65
			0.70	0.72	0.67	0.80
			0.82	0.76	0.94	0.94
			0.83	0.83	0.83	0.88
			0.91	0.89	0.82	0.86
			0.79	0.77	0.76	0.86
			0.72	0.83	0.72	0.83
			0.82	0.84	0.78	0.78
			0.83	0.71	0.74	0.78
			0.89	0.82	0.89	0.83
			0.71	0.79	0.82	0.89
			0.78	0.84	0.88	0.78
		33.6	0.96	0.87	0.91	0.93
			0.96	0.93	0.91	0.91
			0.85	0.91	0.98	0.85
			0.83	0.85	0.85	0.89
			0.86	0.97	0.87	0.92
			0.86	0.88	0.93	0.87
			0.90	0.89	0.97	0.94
			0.99	0.85	0.82	0.90
			0.96	0.90	0.92	0.64
			0.89	0.69	0.88	0.74
			0.81	0.67	0.73	0.69
			0.70	0.75	0.64	0.88
			0.65	0.78	0.70	0.82
			0.65	0.71	0.84	0.67

APPENDIX 2-4. Band sharing coefficients (x100) for two MHC loci calculated from all pair-wise comparisons between adult *T. motierii* on Maria Island. Numbers on the outside of the table identify individual birds. Values above the diagonal are based on blots probed with B-LβII while those below the diagonal are based on blots probed with F10.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		97	94	91	94	91	97	97	97	97	94	97	94	94
2	96		97	94	97	94	100	100	100	100	97	100	97	97
3	96	100		91	94	91	97	97	97	97	94	97	94	94
4	89	92	92		97	100	94	94	94	94	97	94	97	97
5	89	92	92	100		97	97	97	97	97	100	97	100	100
6	92	96	96	96	96		94	94	94	94	97	94	97	97
7	92	96	96	96	96	100		100	100	100	97	100	97	97
8	96	100	100	92	92	96	92		100	100	97	100	97	97
9	92	96	96	96	96	100	100	96		100	97	100	97	97
10	92	96	96	96	96	100	100	96	100		97	100	97	97
11	89	92	92	100	100	96	96	92	96	96		97	100	100
12	89	92	92	100	100	96	96	92	96	96	100		97	97
13	89	92	92	100	100	96	96	92	96	96	100	100		100
14	92	96	96	96	96	100	100	96	100	100	96	96	96	

APPENDIX 2-4 continued.

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
15		93	96	97	96	96	96	96	100	96	96	96	97	96
16	97		96	97	96	96	96	96	97	96	96	96	96	96
17	88	90		93	100	100	100	100	96	100	100	100	93	100
18	100	97	88		96	96	96	96	97	96	96	96	93	96
19	88	90	100	88		100	100	100	96	100	100	100	93	100
20	88	90	100	88	100		100	100	96	100	100	100	96	100
21	88	90	100	88	100	100		100	96	100	100	100	93	100
22	94	97	88	94	88	88	88		96	100	100	100	93	100
23	91	88	90	91	90	90	90	85		96	96	96	97	100
24	94	97	88	82	88	88	88	94	85		100	100	93	100
25	97	100	90	97	90	90	90	97	88	97		100	93	100
26	85	88	97	97	97	97	97	85	94	85	88		93	100
27	97	94	90	97	90	90	90	91	94	91	94	88		93
28	94	97	93	94	93	93	93	94	90	94	97	90	97	

APPENDIX 2-5   Band sharing coefficients (x100) for two MHC loci calculated from all pair-wise comparisons between unrelated adult *T. mortierii* at Geeveston. Numbers on the outside of the table identify individual birds. Values above the diagonal are based on blots probed with B-LβII while those below the diagonal are based on blots probed with F10.

	29	30	31	32	33	34	35	36	37	38	39	40	41	42
29		92	95	92	100	97	100	100	100	95	97	100	92	
30	81		97	100	92	95	92	92	97	97	95	92	95	
31	81	100		97	95	97	95	95	95	100	97	95	97	
32	86	96	96		92	95	92	92	92	97	95	92	95	
33	93	81	81	86		97	100	100	100	95	97	100	92	
34	85	89	96	100	77		97	97	97	97	95	97	95	
35	79	96	96	86	92	100		100	100	95	97	100	92	
36	86	96	96	93	86	92	100		100	95	97	100	92	
37	86	96	96	100	93	92	100	100		95	97	100	92	
38	88	92	92	88	80	96	88	88	88		97	95	97	
39	97	86	86	96	97	81	83	83	83	85		97	95	
40	89	85	92	89	81	96	96	96	96	92	86		97	
41	96	86	85	89	89	88	89	89	89	92	93	92		
42	92	88	88	92	85	92	85	85	85	96	89	88	96	

APPENDIX 2-5 continued.

	43	44	45	46	47	48	49	50	51	52	53	54	55	56
43		95	95	95	95	97	100	97	92	100	100	97	97	97
44	87		95	90	90	92	95	92	93	95	95	92	92	92
45	93	86		95	95	97	95	97	98	95	95	97	97	97
46	94	87	93		100	97	95	97	98	95	95	97	97	97
47	88	93	87	94		97	95	97	98	95	95	97	97	97
48	97	90	90	90	97		97	100	95	97	97	100	100	100
49	94	93	87	94	94	97		97	92	100	100	97	97	97
50	87	93	86	93	93	90	93		95	97	97	100	100	100
51	97	97	90	90	100	100	100	97		92	92	95	95	95
52	83	96	89	90	90	86	90	96	93		100	97	97	97
53	83	96	89	90	90	86	90	96	93	100		97	97	97
54	94	93	87	94	100	97	100	93	100	90	90		100	100
55	94	93	93	94	94	90	94	87	97	90	90	94		100
56	93	86	93	93	93	97	93	93	97	89	89	93	87	

APPENDIX 3-1   Band sharing coefficients (x100) for pair-wise comparisons between all adult individuals on blots probed with the chicken MHC probe B-LβII. Values above the diagonal are for the population of *M. ater* at Delta Marsh while values below the diagonal are for the population of *C. canorus* in Great Britain. Numbers on the outside of the table identify individual birds.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	--	96	92	100	96	96	100	89	96	92	89	92	92	100	100	96	96	92	92
2	57	--	96	96	100	92	96	93	92	89	93	96	89	96	96	92	100	96	96
3	00	00	--	92	96	89	92	97	96	93	97	100	93	92	92	89	96	100	100
4	18	15	00	--	96	96	100	89	96	92	89	92	92	100	100	96	96	92	92
5	04	33	00	44	--	92	96	93	92	89	93	96	89	96	96	92	100	96	96
6	36	31	00	40	89	--	96	93	92	96	86	89	96	96	96	100	92	89	89
7	18	00	25	00	22	20	--	96	96	92	89	92	92	100	100	96	96	92	92
8	71	63	18	15	33	31	15	--	93	90	93	97	90	96	96	86	93	97	97
9	40	00	00	22	25	22	22	33	--	96	93	96	96	96	96	92	92	96	96
10	31	00	20	17	18	17	17	27	73	--	90	93	100	92	92	96	89	93	93
11	18	15	00	20	44	84	20	15	22	17	--	97	90	89	89	93	93	97	97
12	33	29	00	36	40	36	18	43	20	31	55	--	93	92	92	89	96	100	100
13	00	00	50	00	00	00	00	15	22	50	00	18	--	92	92	96	89	93	93
14	20	17	00	22	75	67	22	17	25	18	67	40	00	--	100	96	96	92	92
15	36	46	00	00	44	40	20	46	22	17	40	55	00	44	--	96	96	92	92
16	33	14	22	18	20	36	18	29	40	31	36	17	18	20	18	--	92	89	89
17	16	29	00	55	20	18	00	29	20	31	18	33	00	20	18	00	--	93	93
18	33	14	22	18	20	18	18	43	20	15	36	50	36	40	36	17	17	--	100
19	00	15	00	00	00	00	00	15	22	33	00	18	40	00	20	18	18	00	--