An evaluation of the population biology, genetics and future viability of the breeding Wood Duck (Aix sponsa) population at Arrowwood National Wildlife Refuge
by James Bruce Neill

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biological Sciences
Montana State University
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Abstract:
A breeding population of Wood Ducks (Aix sponsa), introduced to eastern-central North Dakota in 1968 was evaluated. This population occupies habitat outside the native range of Wood Ducks; numbers of breeding females have greatly declined over the past ten years. Two potential factors for this decline in population numbers were evaluated. These are competition from Hooded Mergansers (Lophodytes cucullatus), and reduced genetic variability of the Wood Duck population from the use of captive individuals to seed the population. Analysis of historical nesting data yielded no indication that Hooded Merganser's nesting activities have had any impact on the reproductive success of Wood Ducks. An analysis of 17 polymorphic allozymes was made for the Arrowwood population and a captive population similar to the one originally used to create the Arrowwood population. Using these data, mean heterozygosity, mean number of alleles/locus, and percent polymorphic loci were calculated. All of these indices indicate that the Arrowwood Wood Duck population is more genetically diverse than the captive population, and has levels of genetic variability similar to those reported for other native avian populations. The differences between the two populations were found not to be statistically different. Minisatellite DNA fingerprint analysis was carried out for the Arrowwood population, the captive population and a population from western-central Oregon. These analyses indicate that the Arrowwood population has significantly more variation than the captive population, and both of these exhibit more DNA polymorphism variability than the population in Oregon. It is suggested that the number of nesting females is being underestimated at Arrowwood National Wildlife Refuge because hens are nesting in natural nesting cavities and in areas outside the refuge boundaries. It is suggested that the Wood Duck population in North Dakota has high levels of genetic variability because of a constant influx of novel drakes. Umecorded nesting and high genetic variability suggests the population of Wood Ducks in eastern-central North Dakota is in no danger of immediate extirpation. It appears that Wood Ducks in the Pacific Flyway have experienced long population bottle-necks causing a paucity of genetic variability; further analysis of Pacific Wood Duck populations is suggested.

AN EVALUATION OF THE POPULATION BIOLOGY, GENETICS AND FUTURE VIABILITY OF THE BREEDING WOOD DUCK (*AIX SPONSA*) POPULATION AT ARROWWOOD NATIONAL WILDLIFE REFUGE.

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ABSTRACT

A breeding population of Wood Ducks (*Aix sponsa*), introduced to eastern-central North Dakota in 1968 was evaluated. This population occupies habitat outside the native range of Wood Ducks; numbers of breeding females have greatly declined over the past ten years. Two potential factors for this decline in population numbers were evaluated. These are competition from Hooded Mergansers (*Lophodytes cucullatus*), and reduced genetic variability of the Wood Duck population from the use of captive individuals to seed the population. Analysis of historical nesting data yielded no indication that Hooded Merganser's nesting activities have had any impact on the reproductive success of Wood Ducks. An analysis of 17 polymorphic allozymes was made for the Arrowwood population and a captive population similar to the one originally used to create the Arrowwood population. Using these data, mean heterozygosity, mean number of alleles/locus, and percent polymorphic loci were calculated. All of these indices indicate that the Arrowwood Wood Duck population is more genetically diverse than the captive population, and has levels of genetic variability similar to those reported for other native avian populations. The differences between the two populations were found not to be statistically different. Minisatellite DNA fingerprint analysis was carried out for the Arrowwood population, the captive population and a population from western-central Oregon. These analyses indicate that the Arrowwood population has significantly more variation than the captive population, and both of these exhibit more DNA polymorphism variability than the population in Oregon. It is suggested that the number of nesting females is being underestimated at Arrowwood National Wildlife Refuge because hens are nesting in natural nesting cavities and in areas outside the refuge boundaries. It is suggested that the Wood Duck population in North Dakota has high levels of genetic variability because of a constant influx of novel drakes. Unrecorded nesting and high genetic variability suggests the population of Wood Ducks in eastern-central North Dakota is in no danger of immediate extirpation. It appears that Wood Ducks in the Pacific Flyway have experienced long population bottle-necks causing a paucity of genetic variability; further analysis of Pacific Wood Duck populations is suggested.

Chapter 1

OVERVIEW OF DISSERTATION PROJECT

This thesis presents a study of breeding Wood Ducks (*Aix sponsa*), on Arrowwood National Wildlife Refuge, in east-central North Dakota. The principal focal points of this study are in the areas of conservation biology and wildlife management. It is a multi-disciplinary approach to a single problem of conservation and wildlife management. Information on the breeding biology, behavior, and genetics of this species is compiled to address the viability of an introduced population. In this manner, this study is representative of how successful conservation endeavors must utilize a spectrum of information in order to answer a single question concerning the relative health of populations.

Wood ducks have not traditionally occurred in this region of the United States, and they were purposely introduced to Arrowwood to determine whether or not a breeding population could survive there. Wood Ducks from captive populations were introduced to Arrowwood in 1968. The population flourished for several years and then appeared to decline rather sharply. It was unclear as to why the breeding population of wood ducks was declining at Arrowwood. This study attempts to understand better the apparent decline in breeding Wood Ducks at Arrowwood National Wildlife Refuge and to

analyze the genetics of that population to determine whether the decline could be due to genetic factors in the population arising from its origin as a captive population.

In this investigation, three hypotheses are addressed which could potentially explain the decrease in population of Wood Ducks at Arrowwood. The three hypotheses are : 1) Competition for nesting spaces from Hooded Mergansers is adversely affecting the reproduction of Wood Ducks, 2) Wood Ducks are exposed to environmental toxins at Arrowwood, and the success of Wood Duck reproduction is being adversely affected by exposure to these contaminants, and 3) There is low genetic variability among the Wood Ducks at Arrowwood and this reduced genetic variability is causing a decrease in reproductive success among the Wood Ducks at Arrowwood. By testing these different hypotheses, the overall health and viability of Wood Ducks at Arrowwood National Wildlife Refuge is evaluated.

The thesis is arranged into distinct chapters which cover different aspects of this investigation. The first chapter is an analysis of the population trends of breeding Wood Ducks at Arrowwood; in it an additional evaluation is made to determine whether another waterfowl species, the Hooded Merganser (*Lophodytes cucullatus*), appears to be a contributory factor to the apparent decline of nesting wood ducks. In this chapter, historical nesting data is used to infer whether environmental toxicants appear to be affecting the reproductive success of Wood Ducks. The second chapter is an analysis of the genetic composition of the Arrowwood population and a comparison of that population to a captive population similar to the population from which the Arrowwood population first originated. This genetic analysis uses protein (allozyme), variability to

infer levels of genetic variation within the Arrowwood and captive populations. The third chapter is a genetic investigation based on DNA fingerprinting analyses which measures amounts of genetic variability directly from DNA rather than indirect measurements of proteins. In this chapter, a third population is added to the analysis. This population is a naturally occurring, small population in Oregon. It was analyzed and compared to two populations analyzed in previous chapters.

Through these combined approaches, the three hypotheses proposed are tested and the health and viability of the Arrowwood breeding population of Wood Duck is assessed. This combined approach is indicative of how conservation studies and management practices must draw on a wide variety of techniques to ascertain the viability of natural populations and of management techniques used to alter and sustain populations of wild animals.

Chapter 2

POPULATION ANALYSIS OF NESTING WOOD DUCKS AND HOODED MERGANSERS AT ARROWWOOD NATIONAL WILDLIFE REFUGE.

Introduction

Arrowwood National Wildlife Refuge (ANWR) in eastern-central North Dakota was created in 1935 and contains 15,900 acres of prairie grassland along a 16-mile length of the James River. On the refuge there are four large impoundments of the river producing extensive shallow lakes and marshes; there is very little natural river channel remaining on the refuge. In these extensive limnetic zones there are communities of many aquatic and sub-aquatic plants which provide large areas suitable for waterfowl use.

This area is outside the traditional breeding range of the Wood Duck *(Aix sponsa)*. Wood Ducks were introduced to (ANWR) in 1968 (Doty & Kruse 1972). The initial introduction was accompanied by the placement of approximately 300 nest boxes within the refuge boundaries. These combined activities were parts of an experiment designed to evaluate the effectiveness of establishing Wood Duck populations in novel geographic locations (Doty & Kruse 1972). Since 1969, nest-box utilization has been monitored and recorded on an annual basis by the staff of ANWR as a part of the waterfowl management program, and these data have been used to monitor the status of the breeding Wood Duck population on the refuge.

Wood Ducks faced near extinction in the early parts of this century; it is estimated that in 1915 there were more Wood Ducks in captive flocks in Europe than in the wild in North America (Ripley 1973). This species was saved from extinction by strict legislation imposing a moratorium on hunting (Bellrose & Heister 1987, Baldassarre & Bolen 1994) and considerable re-introduction efforts from European captive populations (Ripley 1973). Since that time, Wood Ducks have made a very successful recovery and are now fairly common throughout their original range even though this species experienced population bottlenecks and perhaps extensive inbreeding for a period of 10-20 generations.

The traditional range of Wood Ducks occupied the Atlantic, Pacific and Mississippi waterfowl Flyways of North America. Within these flyways, Wood Ducks are most abundant in southern regions during both the breeding and winter seasons. Since the 1970's, Wood Ducks have experienced a range expansion and they are now known to breed and winter in the Central Flyway (Ladd 1990); this expansion is attributed to anthropogenic introductions and a natural colonization of the southeastern portions of that area.

Population densities of Wood Ducks are extremely difficult to obtain, and accurate population densities are not available for much of their range. The principal method by which population sizes are estimated is from harvest statistics gathered from hunters. This method does not provide accurate information on actual population numbers, but does provide insight with regard to general population trends. It is perhaps useful to compare numbers between different flyways, although changing harvest regulations and the

dynamic nature of hunting efforts can be confounding factors. Nonetheless, population densities of Wood Ducks appear generally much higher in the Atlantic and Mississippi Flyways than in the Central or Pacific Flyways (Bellrose 1980, Baldassarre & Bolen 1994). An estimate of the number of breeding Wood Ducks for the Central Flyway (Bellrose 1980), was around 50,000 individuals, although this estimate is probably very conservative and could be off by up to 30-50% (May 1986; Ladd 1990). An estimate of the numbers of individuals in the Pacific Flyway is 60,000 (Bartonek *et al.* 1990). Although estimates of absolute numbers of individuals are not available for Atlantic and Mississippi Flyways, harvests in those flyways combined is in excess of 1.2 million individuals per year (Baldassarre & Bolen 1994). From these data, it appears that populations in the Pacific Flyway are the lowest in North America and could be up to two orders of magnitude lower than those of the eastern United States. The origin of Wood Ducks in the Pacific Flyway is unclear, but there has been little or no population mixing between the Pacific Flyway and the flyways east of the Rocky Mountains; this isolation has caused the Pacific populations to remain genetically isolated with respect to other populations.

During the last 20-30 years, Wood Ducks have been successfully introduced to regions outside their native breeding ranges (Doty & Kruse 1973, Baldassarre & Bolen, 1994). Most of the introductions to novel or peripheral environments have been accomplished by using individuals from captive breeding stocks to propagate new breeding populations. One notable introduction was accomplished in 1968, when a

breeding population of Wood Ducks was established in eastern-central North Dakota on Arrowwood National Wildlife Refuge using stocks from captive populations in North Dakota (Doty & Kruse 1972).

Wood Ducks often inhabit densely forested aquatic habitats. These aquatic habitats are extremely difficult ones in which to conduct direct population surveys of this species (Hein 1966, Bellrose 1980, Parr & Scott 1978, Brakhage 1990, Moser & Graber 1990, Robb & Bookhout 1990). Many methods have been employed to make population estimates of Wood Duck abundance, but no feasible method exists to date. Wood Ducks are also very secretive nesters, most commonly nesting in naturally occurring cavities in trees. Such arboreal nesting locations are often difficult to find, and if found they are commonly placed such that access to nests and their contents is impossible to obtain. Wood Duck hens will readily nest in artificial nesting structures, and when nesting in these structures their nesting behaviors are much easier to monitor. Much of the information regarding nesting densities, clutch size, reproductive success, and other reproductive components of Wood Duck biology has been obtained from analysis of nesting activities in artificial nesting structures. Such nest-box monitoring has proved valid to monitor cavity-nesting breeding waterfowl populations, and it is nearly the only method useful for studies of Wood Duck nesting activities (Zicus & Hennes 1987, Ladd 1990, Robb & Bookhout 1988).

The breeding biology of the ANWR Wood Duck population has been evaluated through nest-box data analysis and was presented by Doty *et al.* (1984). During the 13

years following their introduction, the number of nesting Wood Duck females at ANWR has fluctuated widely and appeared to be declining in 1982 (Doty *et al.* 1984). The nesting population experienced a marked increase during the initial years, but in the early 1980's it appeared to be steadily declining, and extirpation was imminent in the near future.

The initial release of Wood Ducks at ANWR consisted of 280 ducklings that had been incubated and reared in the Northern Prairie Wildlife Research Center (Doty & Kruse 1972). The sources for eggs from which these individuals hatched were from captive flocks at the Center and a captive population in Minnesota. After hatching, the ducklings were maintained in the Center for 9 to 16 days and then transported to an open-topped release pen on ANWR; when the ducklings were between 19 and 26 days of age they were sexed, banded and released (Doty & Kruse 1972). Of the 280 initial ducklings released, approximately 253 (132 females and 121 males), survived to flight stages, and approximately 193 survived until all Wood Ducks departed in the fall of 1968 (Doty & Kruse 1972). Band recovery from the first post-release winter indicated that the ducks migrated along the western Central flyway to the western portion of normal Wood Duck winter range (Doty & Kruse 1972). During the following breeding season, 12 of the banded Wood Duck hens from the original release returned to ANWR and nested in nest-box structures on the refuge (Doty *et al.* 1984). For the following six years the number of nesting females continued to increase.

Since the introduction of Wood Ducks to ANWR, Hooded Mergansers (*Lophodytes cucullatus*), have naturally expanded their breeding range to include the aquatic habitats there; in 1973 Hooded Mergansers were first observed to nest at the refuge (Doty *et al.* 1984). Hooded Mergansers are cavity nesters, and it is hypothesized that the two species might compete for nesting cavities and influence one-another's nesting success. Intra- and interspecific cases of nest parasitism are known for each of the species (Morse & White 1969, Clawson *et al.* 1979, Doty *et al.* 1984, Haramis & Thompson 1985, Sherman & Semel 1989). Hooded Mergansers have been reported to initiate nesting activities earlier in the year than Wood Ducks (Fitzner & Fitzner, 1973). This temporal difference in nest-initiation times could allow Hooded Mergansers to exclude Wood Duck hens from nesting structures through a mechanism of exploitative competition. The potential negative influence of Hooded Mergansers to the decline of nesting Wood Ducks at ANWR has been hypothesized by scientists (Doty *et al.* 1984) and by refuge personnel.

When populations are maintained in captivity, there is often a loss of genetic variability due to inbreeding, genetic drift due to low N_e/N ratios and other factors which tend to degrade genetic diversity (Hedrick *et al.* 1986, Briscoe *et al.* 1992). The maintenance of genetic variability is widely believed important because the long-term survival and viability of populations is likely related to levels of genetic variation among members of a population (Soulé 1980, Frankel & Soulé 1981, Barrett & Vyse 1982, Beardmore 1983, Lande 1993). Although the exact mechanisms of this relationship have

been recently questioned (Caro & Laurenson 1994), it is nonetheless widely accepted that populations having small amounts of genetic variability are more extinction-prone than populations with higher levels of genetic variability. In captive breeding programs designed to release individuals into the wild, efforts need to be made to insure that genetic variability is not degraded during captivity so that the individuals released will represent a significant portion of the genetic variability naturally present in the species under management (Hederick *et al.* 1986, Soulé 1987). Many different strategies exist in breeding programs that attempt to minimize degradation of genetic variance in captive populations. This crucial need to manage captive populations for genetic diversity is widely accepted among breeders and game managers now, but it was not recognized at the time of the introduction of Wood Ducks to ANWR in 1968. Consequently, no attention was paid to the genetic composition of the Wood Ducks released at ANWR.

The long-term viability of the Wood Duck population at ANWR is uncertain because: 1) ANWR is outside the traditional nesting range of Wood Ducks, 2) The nesting population is in decline, 3) a strong potential for interspecific nest competition exists, and it originated from captive breeding stocks whose genetic variability is unknown but likely to be below that of natural populations.

In this analysis, nesting data are presented and analyzed to evaluate further population trends of nesting Wood Ducks at ANWR. Nesting success data for both Wood Ducks and Hooded Mergansers are examined to investigate the role of interspecific competition that might adversely affect Wood Duck nesting success.

Materials and Methods

Nesting-box structures on ANWR are monitored every fall (after all nesting activity has ceased) by refuge personnel as part of the waterfowl management program. During this monitoring, each nest-box is located, its condition noted, and the contents of the box recorded. The presence of eggs, egg shells, and/or egg membranes is recorded, quantified, and identified as to the species of origin. The contents of each nest box is then removed, and any repairs and/or modifications needed to the structure or nesting materials are made in preparation for the next nesting season. Hooded Merganser and Wood Duck eggs and egg shells can be readily distinguished from one-another (Soulliere 1985), making it possible to determine which of these two waterfowl species used a particular nest during the past nesting season. From this information, the number of active nests, number of successful nests, and hatching success of eggs is obtained for species.

The original waterfowl nesting data for ANWR was obtained for the years 1968-1988 from the nest-box survey records maintained by the refuge personnel. In 1989, I collected the data for 320 nest box structures at two different times during the year. The first data collection period was during late May when nesting activity was ongoing and Wood Duck hens could be captured on the nests. The second monitoring was made during September after the cessation of nesting activities. The methods I employed were identical to those used by refuge personnel during the previous years.

Results

The numbers of nesting females of both species are presented in Figure 1. There was an increase in the Wood Duck population for the first six years followed by a sharp decline over the next seven years. Since 1982, there has been some variation in the number of Wood Duck nests, but the population is essentially stationary at approximately 20-30 nests per year (Mean = 29 ± 9). The numbers of Hooded Merganser and Wood Duck nests indicate a very general trend of Wood Duck nests decreasing and Hooded Merganser nests increasing (Figure 1). The number of Wood Duck nests does not rapidly decline with the advent of Hooded Mergansers at the refuge, and there is no apparent pattern indicating the size of the breeding population of one species closely correlates with the number of nests of the other species. In 1982, the number of Hooded Merganser nests surpassed the number of Wood Duck nests; and the number of nesting Hooded Mergansers has always been greater then the number of nesting Wood Ducks since 1984. In 1987 both species showed an increased number of nests over the past 3 years.

The number of successful egg-hatches per species per nesting season was used as an index of reproductive success (Figure 2). A regression of egg hatching success over time as expressed as the number of years since the initial nesting of Hooded Mergansers at ANWR revealed that neither species has experienced a significant change in reproductive success over the period of 1979-1989. For Wood Ducks, $r^2 = 0.03$, $F = 0.44$, $p(F) = 0.52$; for Hooded Mergansers $r^2 = 0.02$, $F = 1.13$, $p(F) = 0.29$.

Figure 1. The Numbers of Nesting Females of Hooded Mergansers and Wood Ducks Using Nestboxes at Arrowwood National Wildlife Refuge.

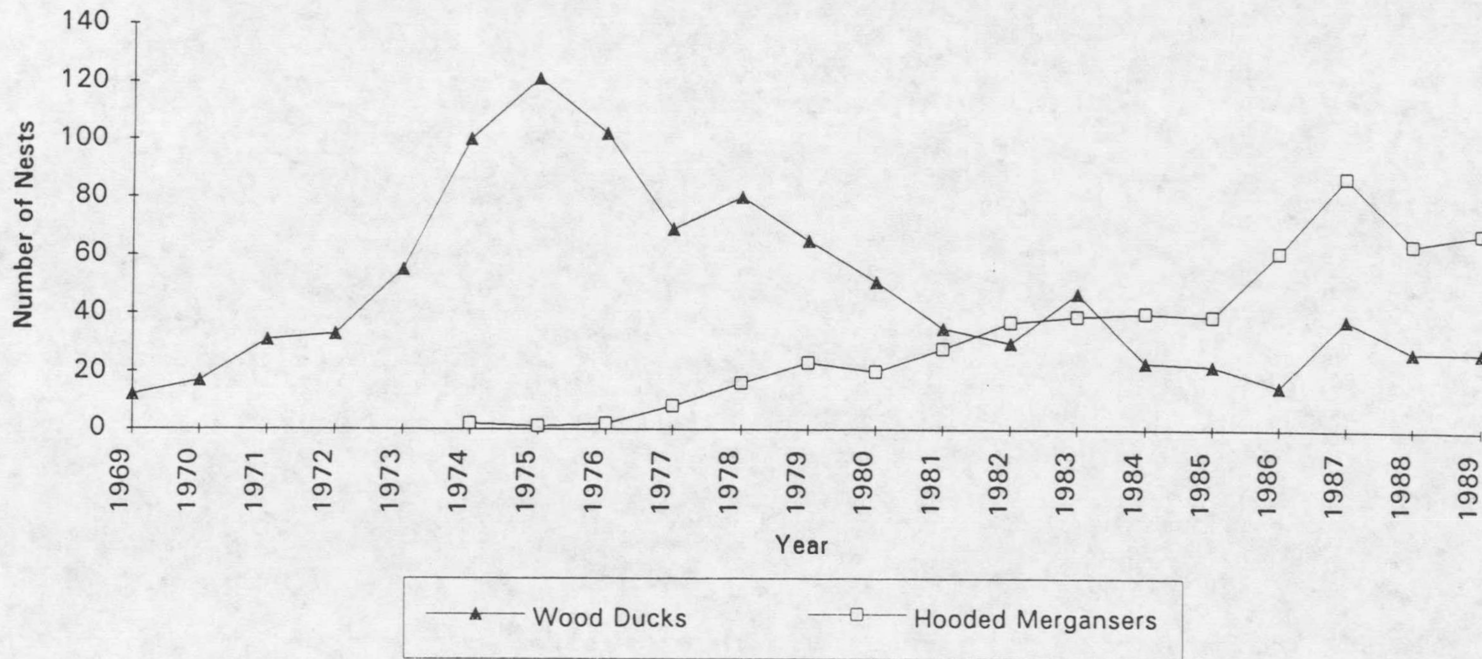
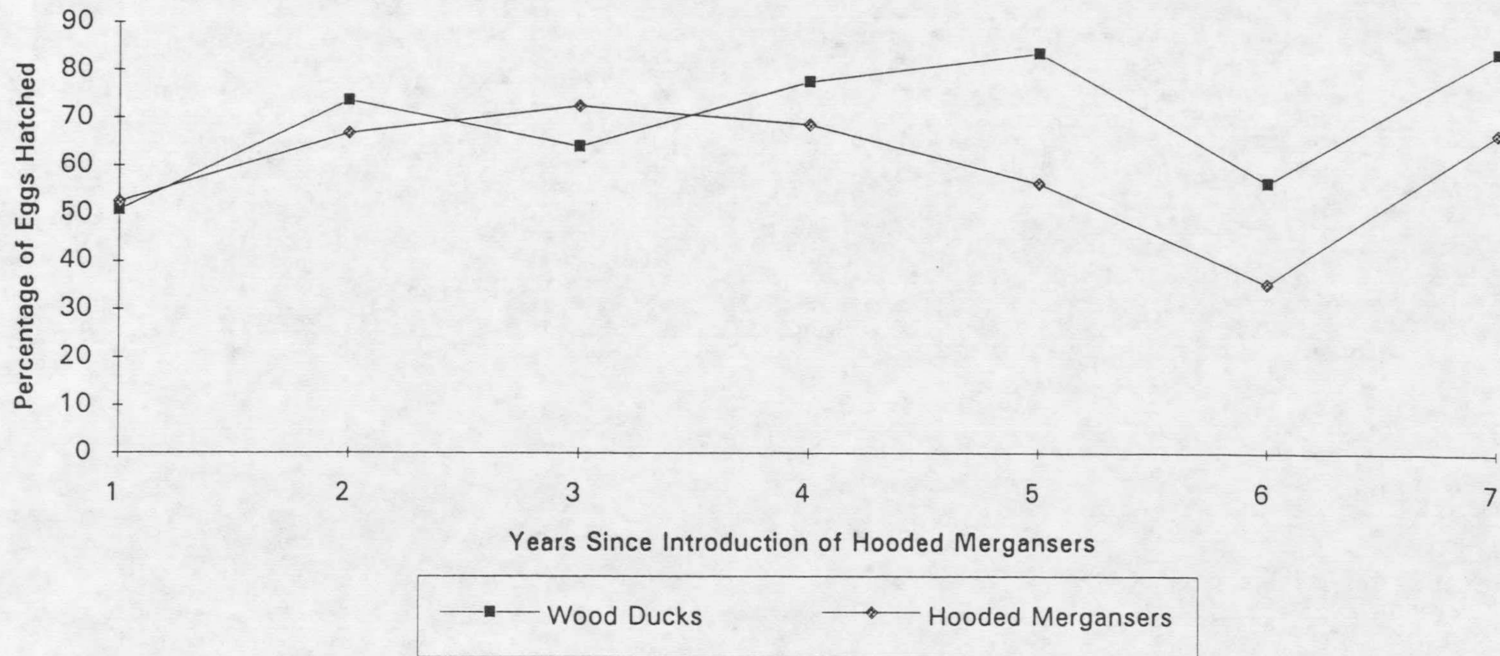


Figure 2. Egg Hatching Success as a Function of Time Since the Introduction of Hooded Mergansers.



A second analysis was carried out by regressing hatching success of one species on the number of nesting females of the other species for the period of 1979-1989. For Wood Duck success as a function of Hooded Merganser nesting population size, $r^2 = 0.2$, $F = 3.64$, $p(F) = 0.08$; for Hooded Merganser success as a function of Wood Duck nesting population size, $r^2 = -0.08$, $F = 0.21$, $p(F) = 0.21$. A significant relationship does not exist between the nesting success of either species and the number of nesting females of the other species.

Table 1. Number of eggs laid, followed by the number of eggs hatched for both Wood Duck and Hooded Mergansers between the years of 1979 and 1989. The percentages in parentheses are the total percentage of eggs hatched per year. Values of G that are not significant at the $\alpha = 0.05$ level are marked with an asterisk.

Year	Wood Duck Eggs-Hatched	Hooded Merganser Eggs-Hatched	G_{adj}
1979	369 - 215 (58.3%)	171 - 103 (60.2%)	0.187*
1980	262 - 147 (56.1%)	163 - 115 (70.6%)	8.980
1981	265 - 208 (78.5%)	238 - 158 (66.4%)	9.244
1982	245 - 174 (71.1%)	150 - 111 (74.0 %)	0.411*
1983	638 - 324 (50.8%)	238 - 453 (52.5%)	0.326*
1984	253 - 344 (73.5%)	355 - 531 (66.8%)	4.445
1985	185 - 288 (64.0%)	350 - 483 (72.5%)	5.677
1986	182 - 235 (77.4%)	489 - 714 (68.5%)	7.079
1987	332 - 398 (83.4%)	388 - 689 (56.3%)	88.292
1988	114 - 202 (56.4%)	232 - 658 (35.3%)	28.3026
1989	319 - 381 (83.7%)	640 - 959 (66.7%)	41.503

To compare reproductive success between the two species, G-tests of independence (Sokal & Rolhf 1981), were carried out for hatching success data between 1979-1989. The number of eggs hatched for each species in each year was used to calculate G and Williams' adjustment was made to obtain a better approximation to the χ^2

distribution (Sokal & Rolhf 1981). The results are that hatching success differed between the two species in seven of the ten years (Table 1). In six of those years, Wood Ducks exhibited higher nesting success than Hooded Mergansers (Figure 2), and only in 1985 did Hooded Mergansers have higher nesting success than Wood Ducks. Hatching success, when viewed as an indicator of overall reproductive success, indicates that Wood Ducks have higher reproductive success per individual than Hooded Mergansers but the number of nesting Wood Ducks is below that of Hooded Mergansers.

To evaluate the possibility that intraspecific competition between nesting females has adversely affected nesting success of either species, a Pearson product-moment correlation coefficient was calculated for the number of eggs laid in a season and the percentage of eggs hatched for each species during that season. In both species, the correlation between number of eggs and hatching success was not significant; for Wood Ducks, $r = -0.27$, $P(r) = 0.414$ and for Hooded Mergansers, $r = 0.14$, $P(r) = 0.68$. This indicates that there was not a significant intraspecific interaction negatively affecting nesting success for either species during the time of this investigation.

Discussion

The number of Wood Ducks using nest boxes on ANWR has apparently declined in recent years. A possible explanation for this decline is that more Wood Duck hens are using natural cavities for nesting and thus go unrecorded as nesting on the refuge. ANWR has many mature cottonwood trees (*Populus deltoides*) surrounding the James River impoundments on the refuge. These trees provide a large number of natural cavities, and

observations have been made of hen Wood Duck activity in and around such cavities by refuge personnel. Studies in other areas have indicated that only 10% of nesting females in an area may utilize nest boxes when natural cavities are available (Soulliere 1985). Further investigation (Soulliere 1990), reveals that in the Mississippi Flyway there is a latitudinal trend of nest-box utilization among Wood Ducks; in northern latitudes, Wood Duck hens use nest-box structures less commonly than in southern latitudes. One hypothesis proposed to explain this phenomenon is that it is density-dependent and that at lower population densities nest boxes are used less frequently. Wood Ducks are very secretive nesters, and it is difficult to survey and quantify nests in natural nesting cavities (Brakhage 1990, Cottrell & Prince 1990, Sauer & Droege 1990). However, the presence of hens in and around natural nesting cavities suggests that unmonitored nesting is occurring on the refuge.

Since the time of Wood Duck introductions to ANWR, several nest-box programs have been instigated on private lands near the refuge. The refuge may no longer contain the entire breeding population of Wood Ducks in eastern-central North Dakota, and thus the decline in nest box use within the refuge boundaries may reflect this. The existence of non-refuge nesting is suggested by information gathered during late summer wood-duck trapping programs on the refuge. Cannon nets are deployed over artificial baits in the early fall; the netted birds are banded, and their sex and age determined. Specific hatching areas of the young-of-the-year are unknown, but capture numbers are often too high to be accounted for by the number of Wood Duck nests on the refuge. These surplus young-of-

the-year are probably produced in nests in natural cavities and/or nest boxes outside the refuge boundaries but in the vicinity of the refuge.

Analysis of egg-hatching data for the two species at ANWR reveals that Wood Ducks commonly have a higher egg-hatching success rate than Hooded Mergansers, and that hatching success has not significantly changed from 1979-1989. The constancy of egg-hatching success indicates that the reproductive success per female has not changed during the decline of Wood Duck nesting at ANWR. The values of egg-hatching success at ANWR falls within the boundaries reported elsewhere for native Wood Duck populations (Clawson *et al.* 1979, Haramis & Thompson 1985, Semel *et al.* 1988). Additional analysis reveals that hatching success of one species at ANWR is not significantly related to the nesting density of the other species. Although the presence of a weak negative relationship between Wood Duck nesting success and the number of Hooded Mergansers nesting in nest boxes ($r^2 = -0.2$, $F = 3.64$, $p(F) = 0.08$) may be indicative of some interaction between the two species that negatively impacts Wood Duck nesting success the weak nature of this relationship suggests that this interaction is likely not the driving factor behind Wood Duck nesting declines at ANWR. A similar pattern of increasing Hooded Merganser nesting and concomitant decreasing Wood Duck nesting has been reported from Maine by Allen *et al.* (1990); but they found no evidence that would suggest the Hooded Merganser increase was a contributory factor to the decline of nesting success of Wood Ducks.

Wood Ducks have a higher reproductive success than Hooded Mergansers, but numbers of nesting Wood Ducks are declining at ANWR. These differences may be a function of high winter mortality in Wood Ducks. Wood Ducks are highly sought after by hunters and often represent the third most commonly harvested duck species in the U.S., comprising up to 10% of the total U.S. duck harvest by hunters (Bellrose & Heister 1987, Baldassarre & Bolen 1994). Annual hunting harvests of Wood Ducks steadily increased from 1966 through 1985, and 1.23 million individuals were harvested per year during the period of 1981-1986 (Baldassarre & Bolen 1994); approximately 98% of those harvests are from the Atlantic and Mississippi Flyways. Harvest rates have declined since 1986 (Baldassarre & Bolen 1994); such harvest rates probably reflect decreases in total Wood Duck populations in the U.S. Hooded Mergansers are commonly not a prime target species and as such have a much reduced hunting-induced mortality (Baldassarre & Bolen 1994). This disparity in winter hunting harvests may account for declining number of nesting Wood Ducks and a concomitant increase in Hooded Merganser nests.

Another factor possibly accounting for declining Wood Duck populations is post-hatching duckling survival. Wood Ducks and Hooded Mergansers utilize different brood-rearing habitats (Bellrose 1980, Kirby 1990, Baldassarre & Bolen 1994); Wood Ducks utilize marshy, lotic regions along shorelines whereas Hooded Mergansers utilize limnetic zones. Such habitat utilization differences could contribute to variable survival rates of the two species during the nesting season. However, no evidence suggests that hatchling

habitat used by Wood Ducks has been altered or degraded at ANWR since the time of initial introductions on the refuge.

Two additional factors that could contribute to a decline in Wood Duck numbers at ANWR are low genetic diversity resulting from founder effects of the initial release of pen-raised individuals or the presence of environmental contaminants on the refuge. Others sections of this investigation report on levels of genetic diversity in this populations and compare it to captive populations and published reports for other waterfowl species. These results indicate that genetic diversity is not low among ANWR nesting Wood Ducks as compared to other avian species.

Wood Ducks are known to experience reproductive impairment when contaminated by dioxins commonly associated with agri-chemicals (White & Seginak 1994). The areas surrounding ANWR are predominantly agricultural lands, and the decreased flow of the James River through the impoundments on the refuge would allow for an accumulation of agricultural toxins in these waters. When Wood Duck are exposed to dioxins, egg-hatching success is decreased (White & Seginak 1994). The relatively high egg-hatching success of Wood Ducks at ANWR and the constancy of this success over time indicates that these birds are probably not heavily influenced by environmental toxins on the refuge. Wood Duck nesting success decreases at high nesting densities due to antagonistic intraspecific behavioral interactions between nesting females and nest-parasitism (Fellman 1993, Semel *et al.* 1988). Hooded Mergansers could easily be subject to such reduced reproductive efficiency at elevated nesting densities as they are known to

be intraspecific nest parasites. Hooded Merganser nests are parasitized by both intra- and interspecific (Wood Duck) females at ANWR. However, the average egg-hatching success found for ANWR mergansers from 1979-1989 is within the range reported elsewhere for this species (Allen *et al.* 1990, Zicus 1990). The high breeding success of Hooded Mergansers at ANWR may be ephemeral if the population continues to grow; density dependent factors such as interference competition may limit Hooded Merganser breeding population numbers in the future.

Both species appear to have experienced rapid population increases immediately following colonization of the breeding habitat at ANWR. The population fluctuations suggest the presence of a founder-flush pattern of population change following a novel introduction. During the early stages of population establishment, high nesting densities are found for both species. The philopatric nature of both species, along with their high fecundity could account for rapid initial population increase and accompanying increases in competitive interactions that would influence nesting activities. A possible result of such interactions could be females utilizing alternative nesting sites, such as natural cavities. The Wood Duck nesting population data (Figure 1), indicate that they are beyond the effects of a founder-flush population growth phase and are becoming more stationary in population size. The Hooded Merganser is still likely to be under the influence of a founder-flush population change and will perhaps become more stationary in the future.

Although the number of nesting Wood Duck females has declined in recent years at ANWR, there does not appear to be evidence that Wood Ducks are in danger of extirpation in eastern-central North Dakota. While the numbers of Wood Ducks nesting in nest-box structures within the refuge boundaries has declined, evidence indicates that a viable population of Wood Ducks now occurs in this region. The refuge has probably acted as a population center from which dispersal of nesting females has occurred. The original release of Wood Ducks in this geographic region has facilitated a range expansion.

Chapter 3

AN INVESTIGATION OF ALLOZYME VARIABILITY IN A CAPTIVE AND AN INTRODUCED POPULATION OF WOOD DUCKS.

Introduction

Heritable genetic diversity is an important component of a species' ability to persist over time. The presence of phenotypic variance provides the raw material requisite for natural selection and evolution to occur. The importance of phenotypic variability to the process of evolution has been understood since the time of Darwin, and genetic diversity is widely thought at least to reflect and probably provide much of the phenotypic variability observed among individuals. Genetic variance provides the raw material upon which the mechanism of evolution (natural selection and differential reproduction) acts, thus allowing species to persist in the face of dynamic environmental conditions over substantial periods of time.

Studies of population biology and genetics have provided a rich knowledge of the amounts of genetic variation in populations and how various factors affect the amount of genetic variability among individuals of populations. From these studies, several factors emerge as significant in reducing levels of genetic variation; these include small effective population sizes, founder effects, and prolonged (more than 4 to 5 generations), population bottlenecks. These factors tend to erode levels of genetic diversity and thus

decrease the likelihood of a population in adaptation and survival over significantly long (evolutionary) periods of time.

Since the early 1970's, allozyme variation has been a standard technique to determine levels of genetic variability in almost all taxa, and allozyme research revealed patterns and trends in the distribution of genetic variation among widely differing taxa. One trend is that vertebrates exhibit much lower amounts of allozyme variability than do invertebrates (Powell 1975, Selander 1976). In many cases vertebrates have about half the allozyme variability of invertebrates (Nevo *et al.* 1984, Evans 1987). Among vertebrates, larger-bodied species tend to exhibit lower amounts of genetic variability than do smaller species (Nevo *et al.* 1984). An exception to this general pattern among vertebrates is found within the birds; birds exhibit reduced levels of allozyme variability in comparison to most other homeothermic vertebrates regardless of body size (Nevo 1978, Nevo *et al.* 1984, Cooke & Buckley 1987). In fact, many large-bodied avian species exhibit more genetic variation than do smaller-sized birds (Evans 1987). This reduced level of variability among birds is thought to reflect historical events such as inbreeding and or repeated and persistent population bottlenecks that counteract social and behavioral actions that would have increased or at least maintained levels of genetic variance (Evans 1987).

Recently, there has been an increased interest in breeding programs that attempt to maintain individuals of rare or endangered taxa in captive situations. Such breeding programs are now widely considered to be an essential component of management and

preservation of local and global biotic diversity (Foose 1983). Captive propagation is an effective method for producing and maintaining large numbers of individuals to re-stock populations that are either rare or have become extirpated. In fact, for very rare species, captive propagation may be the only method of restoring populations to the wild; this has been the case in Wood Ducks (Ripley 1973, Baldassarre & Bolen 1994), the Black-footed Ferret (Thorne *et al.* 1988, Thorne & Oakleaf 1991) and several other species that have experienced population comebacks through concerted conservation efforts.

In many captive breeding programs, the taxa being bred and maintained are often large vertebrates, birds, or other taxa with low amounts of genetic variability. One of the chief concerns of maintaining captive populations is the retention of genetic diversity over the period of captivity (Soulé & Wilcox 1980, Foose 1983, Ralls & Ballou 1986, Hederick *et al.* 1986, Soulé 1987). The maintenance of such diversity in captive populations is often difficult as they are usually small, having experienced severe population bottlenecks, and may already be highly inbred. All of these factors are known to contribute to losses of genetic diversity in captive populations (Chapco *et al.* 1973, Sing *et al.* 1973, Rumball 1974, Mina *et al.* 1991). Even in large captive populations, genetic variance may decrease over time due to low N_e/N ratios and reproductive demographics differing from those in wild situations (Foose 1983, Lande 1993, Briscoe *et al.* 1992).

Wood Ducks were introduced to Arrowwood National Wildlife Refuge in 1968 as an experiment to evaluate the feasibility of creating populations of this species outside its native breeding range. During the first breeding season, this population was composed of

twelve hens. Those individuals had been released the previous year from captive Wood Duck flocks (Doty & Kruse 1972). The Wood Duck population at ANWR flourished for a period of time and then began to decline at a constant rate, and it appeared that extirpation was likely (Doty *et al.* 1984). At the time of the introduction, the importance of genetic variability, and the likelihood that captive populations have reduced amount of genetic variability was not widely recognized and was not considered. One of the factors thought to be influential in the decline of the breeding Wood Duck population at ANWR is reduced genetic variability due to founder effects associated with the small number of individuals introduced from captive stocks.

In this chapter, allozyme variability was investigated using tissue samples collected from Wood Ducks in a non-destructive fashion from two populations. These populations include the population at ANWR, and a captive population (Hancock), which serves as a representative of the original captive stock used to create the ANWR population. The captive population used as a source of colonists for ANWR is no longer in existence, but the Hancock population is a close approximation of that original population in size and longevity of captivity. The Hancock population is composed of about 12 breeding hens and anywhere from 5 to 25 drakes; it is maintained in eastern Montana and has been in captivity for approximately 12 years (B. Hancock, pers. comm.).

Materials and Methods

Proteins were extracted from samples of blood and/or tissue pulp. Emerging feather quills (blood quills), and blood were collected in the field and immediately stored under cryogenic conditions until transported to the laboratory for preparation and analysis. All blood quill samples were stored at -80° degrees C until protein extraction and electrophoretic analysis. Feather-quill tissues were collected, stored and prepared essentially following the methods of Marsden & May (1984). Feather tissues were homogenized by crushing them with a micro-pestle in a microfuge tube in 500 μl of cold extraction buffer (0.05 M Tris-HCl, 0.05 M Tris-Base, pH 7.1) on ice. Homogenates were centrifuged at 12,000 rpm for a period of 5 minutes and either immediately electrophoresed or stored at -80° C until electrophoresis was carried out. Blood samples were collected by venous puncture of the brachial vein using a 21 gauge needle on a 1 ml hypodermic syringe; 0.5 - 1.0 ml of blood was collected. The blood was placed in a microfuge tube containing 1.0 ml 0.9% saline and 0.1 % sodium citrate to act as an anti-coagulant (Cooke & Buckley 1987). Buffered blood samples were kept at approximately 4° C until prepared for freezing and enzyme extraction. Blood sample preparation was always carried out within 16-30 hours of collection. Whole blood samples were centrifuged at 2000 rpm for 3-5 minutes; the supernatant was discarded, and the pelleted red blood cells were washed 3x with 0.9 % saline and then lysed with an equal volume of distilled water. Blood samples were then either directly applied to electrophoretic gels or stored at -80 °C until electrophoresis was carried out.

A total of 34 loci were preliminary tested for resolution during early phases of electrophoretic analysis. Of the 34 screened, 28 presumptive allozyme loci were found to be of sufficient resolution to be assayed for allelic variation using horizontal starch-gel electrophoresis (Table 2). Electrophoresis and enzyme staining were carried out following the methods of May *et al.* (1979), with modifications described in Britten & Brussard (1992). The enzymes analyzed and the specific buffer systems used to resolve them are listed in Table 3. "R" buffer is from Ridgeway *et al.* (1971), "4" buffer is from Selander *et al.* (1971), and "9C" buffer is from Cooke & Buckley (1987). Protein samples were applied to starch gels using filter-paper wicks saturated with homogenate supernatant; wicks were left in place for a period of 20-30 minutes after the start of electrophoresis and removed after the samples had adsorbed into the gels. Electrophoresis was carried out for a period of four to six hours, depending on specific buffer systems; gels were kept cold during electrophoretic runs. Genotype determination was inferred by direct visual analysis of the gels after specific enzyme staining. Any genotypes that were not readily scorable were tentatively assigned a genotype and then re-run in subsequent electrophoretic runs for clarity of genotype determination. Known samples were included as controls in all electrophoretic runs so that genotype determination could be made with a high degree of certainty.

Table 2. Symbols, names, Enzyme Commission Number used for allozyme analysis of *Aix sponsa*.

Symbol	Enzyme Name	E.C. Number
AAT	Aspartate aminotransferase	2.6.1.1
ACP	Acid Phosphatase	3.1.3.2
ALB	Albumin	
ALD	Aldolase	
EST-1,2	Esterase	3.1.1.1
GAPDH	Glucose-6-phosphate Dehydrogenase	1.1.1.49
GPI	Glucose Phosphate Isomerase	5.3.1.9
Hb-1,2	Hemoglobin	
HBDH	Hydroxybutyric Dehydrogenase	
IDH-1,2	Isocitrate Dehydrogenase	1.1.1.42
LDH-1,2	Lactate Dehydrogenase	1.1.1.27
MDH-1,2	Malate Dehydrogenase	1.1.1.37
MPI	Mannose Phosphate Isomerase	5.3.1.8
ODH-1,2	Octonal Dehydrogenase	1.1.1.73
PEP-LA-1,2	Peptidase-C (Leucyl-alanine)	3.4.11/13
PEP-GL	Peptidase-glycyl-leucine	
PEP-LGG	Peptidase-B (Leucyl-glycyl-glycine)	3.4.11/13
PEP-LLL	Peptidase-leucyl-leucyl,leucine	
PDG	6-Phosphogluconate Dehydrogenase	1.1.1.44
XDH-1,2	Xanthine Dehydrogenase	1.2.1.37

The FORTRAN program BIOSYS-1 (Swofford & Selander 1981), was used to analyze the genotypic frequencies obtained from the gels. The following indices of genotypic variation were analyzed: mean heterozygosity per locus (H), calculated as the proportion of individuals that are actually heterozygous (the "direct-count method" of Swofford & Selander 1981). Estimates of mean heterozygosity per locus were also calculated based on Hardy-Weinberg equilibrium predictions; methods that include sample

size biases and unbiased estimates were calculated (Selander & Swofford 1981). The average number of alleles/locus was calculated as the total number of alleles over all loci. The proportion of polymorphic loci (P), was calculated using the criterion of considering a locus polymorphic when the frequency of the most common allele is < 0.99. Deviations from Hardy-Weinberg equilibrium predictions were examined by calculating exact significance probabilities to overcome the difficulties of small sample sizes associated with the Chi-squared distribution (Sokal & Rolhf 1981). The Fixation Index (F_{IS}), was calculated to analyze patterns of deviations from Hardy-Weinberg equilibrium conditions (Wright 1965, 1978, Nei 1977). Statistical comparisons of genetic indices were made utilizing the computer package SIGMA-STAT.

Table 3. Buffer systems and types of tissue samples used to resolve the loci under investigation. Buffer identification are given in text. For tissues, Q refers to feather quill tissue and R denotes red blood cell samples.

Enzyme	Buffer System	Tissue
AAT	C	Q
ACP	C	Q
ALB	4	R
ALD	4	R/Q
EST-1,2	9C	Q
GAPDH	C	R
GPI	4	Q
Hb-1,2	9C	R
HBDH	9C	Q
IDH-1,2	C	R
LDH-1,2	C	R
MDH-1,2	4	Q

Table 3 (Continued). Buffer systems and types of tissue samples used to resolve the loci under investigation. Buffer identification are given in text. For tissues, Q refers to feather quill tissue and R denotes red blood cell samples.

Enzyme	Buffer System	Tissue
MPI	9C	R
ODH-1,2	C	Q
PEP-LA-1,2	C	Q
PEP-GL	9C	Q
PEP-LGG	C	Q
PEP-LLL	9C	Q
PDG	9C	Q
XDH-1,2	C	Q

Results

Of the 28 loci resolved for *Aix sponsa*, 17 were found to be polymorphic for both populations (Table 4). The invariant loci in both populations were LDH-1,2, Hb-1,2, GAPDH, IDH-1, AAT-2, ACP-1, ODH-1,2, HBDH, XDH-1,2, and ALD. Four other loci were found to be invariant in one population, but polymorphic in the other. The loci with private alleles in the ANWR population are Est-2, GPI, MDH-1, while IDH-2 exhibited an allele in the Hancock population that was not sampled in the ANWR population.

Mean heterozygosity per locus (H), values are given in Table 5. Using a direct-count criterion, H was 0.032 (SE 0.015) in the Hancock population and 0.045 (SE 0.012) in the ANWR population. A t-test was carried out to ascertain whether H values were significantly different; this analysis revealed no significant differences between the ANWR

and Hancock direct-count values of H (t = 0.908, df = 53, P = 0.368). The direct-count method of calculating H was compared to estimates based on Hardy-Weinberg equilibrium predictions (Swofford & Selander 1981) using a Mann-Whitney Rank Sum test; none of the values of H differed from one-another significantly in either population (Table 5).

The mean number of alleles per locus was 1.32 (SE 0.12) for the Hancock population, while the ANWR population had a mean of 1.68 (SE 0.15) alleles per locus (Table 5). A t-test indicated that there is not a significant difference between the mean number of alleles/locus of these two populations (t = -1.84, df = 54, P = 0.07).

Table 4. Allelic frequencies for all loci from Arrowwood National Wildlife Refuge and from a captive population (Hancock) of wood ducks.

Locus	Allele	Hancock	ANWR
ATT	A	1.0 (33)	1.0 (15)
ACP	C	1.0 (33)	1.0 (27)
ALB	C	0.987 (38)	0.934 (38)
	B	0.013	0.066
ALD	C	1.0 (33)	1.0 (37)
EST-1	C	0.811 (37)	0.962 (39)
	D	0.189	0.038
EST-2	C	1.0 (37)	0.976 (42)
	B		0.024
	D		0.073
	E		0.024
GAPDH	C	1.0 (37)	1.0 (41)
GPI	C	1.0 (33)	0.976 (42)
	B		0.024
Hb-1	C	1.0 (37)	1.0 (42)
Hb-2	C	1.0 (37)	1.0 (42)
HBDH	C	1.0 (33)	1.0 (40)
IDH-1	C	1.0 (33)	1.0 (39)
IDH-2	C	0.985 (33)	1.0 (39)
	B		0.015
LDH-1	C	1.0 (37)	1.0 (42)
LDH-2	C	1.0 (37)	1.0 (42)

Table 4 (Continued). Allelic frequencies for all loci from Arrowwood National Wildlife Refuge and from a captive population (Hancock) of wood ducks.

Locus	Allele	Hancock	ANWR
MDH-1	C	1.0 (33)	0.988 (42)
	B		0.012
MDH-2	C	1.0 (33)	0.988 (42)
	D		0.012
MPI	C	0.987 (38)	0.976 (42)
	D	0.013	0.012
	B		0.012
ODH-1	C	1.0 (33)	1.0 (40)
ODH-2	C	1.0 (33)	1.0 (40)
PEP-LLL	C	1.0 (33)	0.913 (23)
	D		0.043
	B		0.043
PGD	C	1.0 (33)	0.551 (39)
	D		0.192
	B		0.256
XDH-1	C	1.0 (33)	1.0 (43)
XDH-2	C	1.0 (33)	1.0 (43)
PEP-GL1	C	0.939 (33)	0.902 (33)
	D	0.015	0.073
	B	0.015	0.025
PEP-LA1	C	1.0 (33)	0.936 (33)
	D		0.013
PEP-LA2	C	0.712 (33)	0.863 (40)
	B	0.106	0.038
	D	0.182	0.100
PEP-LGG	C	0.955 (33)	0.936 (39)
	B	0.045	0.064

The proportions of polymorphic loci (P) for the two populations are given in Table 5. Values are given using three different criteria for designating a locus as polymorphic. There is substantial difference in each population between the 0.95 and the 0.99 criteria. A G-test for independence (Sokal & Rolhf 1981), was carried out to test for significant

differences between the two populations using a criterion of the most common allele having a frequency of < 0.99. The original locus frequencies of monomorphic and polymorphic loci were used to calculate the G statistic. This analysis reveals that the difference between the two populations is not significant ($G = 2.75$, $df = 1$, $0.05 > \alpha < 0.1$).

Analyses of deviations from Hardy-Weinberg expectations revealed that all but 5 loci were within Hardy-Weinberg predictions. Of the five, only PEP-LA-2 was found to be outside of equilibrium conditions in both populations. Two loci were out of Hardy-Weinberg equilibrium for the Hancock population (Pep-LA-2 and Est-1), and four loci deviated from Hardy-Weinberg expectations in the ANWR population (Pep-LLL, Pep-LA-2, PEP-LGG, and GPI). The Fixation Index (F_{IS}), values for the two loci out of Hardy-Weinberg equilibrium in the Hancock population were 0.119 and 0.257 for Est-1 and Pep-LA-2 respectively, indicating a heterozygote deficiency for each of these loci. The F_{IS} values for the ANWR loci deviating from Hardy-Weinberg were 0.198 (Pep-LLL), 0.183 (Pep-LA-2), 0.359 (Pep-LGG), and 0.784 (GPI). As in the Hancock population, these values indicate a paucity of heterozygotes sampled with respect to the predicted values.

Table 5. Mean heterozygosity (H), Mean numbers of alleles/locus, and percent of polymorphic loci (P) for the two populations (ANWR and Hancock), under investigation. The values in parenthesis are the standard error of the mean.

Genetic Index	Population	
	ANWR	Hancock
H		
Biased Estimate[1]	0.064 (0.023)	0.037 (0.019)
Unbiased Estimate[1]	0.065 (0.024)	0.038 (0.020)
Direct-Count Method	0.045 (0.012)	0.032 (0.015)
Mean Alleles/Locus	1.680 (0.150)	1.320 (0.120)
P		
95 % Criterion	25.0	10.7
99 % Criterion	46.4	25.0
No Criterion	46.4	25.0

[1] - See Swofford & Selander (1981), for a further description of biased and unbiased estimates of H.

Discussion

Mean heterozygosity values obtained for Wood Ducks in both populations fall within the range reported for other avian species (Barrowclough 1983, Cooke & Buckley 1987, Gavin *et al.* 1991). In a survey of avian allozyme analyses Cooke & Buckley (1987), report an overall mean heterozygosity value of 0.044 for 86 avian species (H values for individual species ranged from 0.002-0.147). These values are slightly lower than mean heterozygosities (H=0.049), reported for non-avian vertebrates (Evans 1987). Few allozyme investigations exist for waterfowl species to use as comparisons for these results; of the allozyme information available, most studies on waterfowl have been on

Mallards (*Anas platyrhyncos*) and closely related sibling species. Mean heterozygosity values for Mallards range from 0.015 (Browne *et al.* 1993), to 0.076 (Ankney *et al.* 1986); an average of reported values for mallards is 0.046 (Ankney *et al.* 1986, Browne *et al.* 1993). Other mean heterozygosities range from 0.014 for the Laysan Duck (*A. laysanensis*), 0.035 for the Hawaiian Duck (*A. wyvilliana*), (Browne *et al.* 1993), and 0.053 for the American Black Duck (*A. rubripes*), (Ankney *et al.* 1986). Average heterozygosities for Wood Ducks fall in the middle of reported values for other waterfowl species, but ANWR values are more similar to those for Mallards, whereas Hancock populations are more similar to those of the Hawaiian Duck. Mallards have much higher population sizes, and have been exposed to fewer population bottlenecks than the Hawaiian Duck. One would expect an open population such as ANWR to exhibit higher levels of heterozygosity than the Hancock population which is smaller, has a greater likelihood of being inbred and has reduced levels of gene flow. Barrett and Vyse (1982), report mean heterozygosity values for Trumpeter Swans at 0.009; these values are much lower than those found for Wood Ducks in this investigation. Trumpeter Swans are known to have experienced long periods of population bottlenecks and exhibit reduced levels of heterozygosity.

The percent of polymorphic loci was found to be 46.4% for ANWR and 25.0% for Hancock. Reported values range between 26.4% to 22.4% for Black Ducks and Mallards, respectively (Ankney *et al.* 1986); while 5.0% and 17.5% are reported for Laysan and Hawaiian Ducks (Browne *et al.* 1993). Both of these are similar to the findings of Avise

et al. (1990a), for wintering populations of Mallards. The value for the Hancock population is very close to that of Black Ducks and Mallards, while the ANWR population is much higher than any reported values for duck species. In fact, the P values in the ANWR population are among the highest reported for avian species (Cooke & Buckley 1987). Nevertheless, a number of bird studies have reported still higher levels of loci polymorphic (Baker & Manwell 1975, Smith & Zimmerman 1976, Yang & Patton 1981), however, making the ANWR estimates within reason. A loss of polymorphisms is commonly attributed to a low N_e common in many captive populations, (Chesser 1983). The Hancock population would be expected to have a much lower N_e than the ANWR population due to decreased numbers of immigrant hens. This low N_e and isolation from migrants would prevent the influx of new alleles into the population, thus decreasing the levels of polymorphism through the loss of rare alleles through genetic drift.

Statistical comparisons between Hancock and ANWR genetic indices yield a consistent pattern of non-significant differences between the two populations, yet for all indices, the Hancock population consistently exhibited less genetic variability than did the ANWR population (Table 4). The most likely explanation for this lack of significance comes from the low degrees of freedom associated with comparisons between two populations; it is often very difficult to attain significant differences when comparing two population estimates. It is probably safe to assume that the consistent pattern of lower genetic variance in the Hancock population is a meaningful trend while not being statistically significant. It is not a common practice to make statistical comparisons of

genetic indices for different populations; this may result from the difficulty of obtaining statistical significance in such comparisons.

Assuming the Hancock population is representative of the original captive population used to propagate the ANWR population, the proportion of polymorphic loci exhibited in the ANWR population indicates that this population has experienced an increase in genetic diversity since the time of its initial introduction. This is perhaps not what would be predicted in a closed population introduced to the peripheral regions of a species range. Studies on the Common Myna (*Acridotheres tristis*), in Hawaii have shown that although mean heterozygosity did not change after introduction, the proportion of polymorphic loci appeared to decrease (Fleischer *et al.* 1991). This loss of polymorphisms without a significant loss of heterozygosity is predicted by theoretical considerations and is supported in other evaluations of genetic variability (Nei 1977, Falconer 1981, Hedrick 1983, Evans 1987). Examination of the mate selection behaviors of *A. sponsa* provides a mechanism through which an isolated, peripheral population could gain genetic diversity through outbreeding, making it an open rather than a closed population. In Wood Ducks, mate selection occurs by hens selecting drakes on the winter range. During the winter months, there is a concentration of Wood Ducks in the southern United States; all of the Wood Ducks in the Central flyway aggregate in the near-coastal waters of the Mississippi River drainage of Louisiana, Texas, and Mississippi. This provides the opportunity for females to recruit new males into the population, and the entire Central Flyway may act as one large, effectively panmictic, population. Similar panmixis is known to occur for other

vertebrates that disperse widely to reproduce and after mate selection and fertilization occurs in large aggregations (Avise *et al.* 1990b). Effective panmixia is also reported for the sessile invertebrate *Tridacna gigas* in apparently isolated populations on the Great Barrier Reef off Australia; the long-range dispersal mechanism is thought to facilitate gene flow among these isolates. The presence of panmictic mating in Wood Ducks in the Central Flyway would provide a mechanism for the maintenance of high levels of polymorphism in small breeding units such as ANWR.

Observed deviations from Hardy-Weinberg equilibrium can result from a variety of factors affecting populations (Hedrick 1983). One such factor that has been highly debated is whether the characters examined are under the influence of natural selection. A recent review of allozyme studies (Watt 1994), provides evidence that some loci used in allozyme studies do exhibit evidence of being under strong selective forces, but when considered in large numbers, it is probably safe to discount natural selection as being the driving force in maintaining non-equilibrium in these populations. A second source of non-equilibrium genotypic frequencies is that of inaccurate genotype scoring of gels, rather than some natural phenomenon acting on populations. Because only one locus (Pep-LA-2), was found to be outside equilibrium in both populations scoring errors do not appear to be solely responsible for the observed deviations from Hardy-Weinberg equilibrium, or if they are the scoring errors occurred equally for both populations. Browne *et al.* (1993), report Pep-LA to be out of Hardy-Weinberg equilibrium for Mallards, and Hawaiian Ducks; other Peptidase loci have been found to be outside of

Hardy-Weinberg equilibrium in non-waterfowl avian species (Rasmussen 1994). This constancy of non-equilibrium could indicate that selective pressures could be acting on this particular locus to maintain it outside of Hardy-Weinberg equilibrium in a variety of avian species, or could be indicative of the presence of null alleles in many avian species. A third factor affecting Hardy-Weinberg equilibrium is inbreeding (Falconer 1981). Inbreeding is common among captive populations (Chesser 1983, Soulé 1987) and it could result in heterozygote deficiencies. However, an unexpected pattern of equilibrium deviation was found; the natural population was less frequently in Hardy-Weinberg equilibrium than the captive population. Sampling of genetic neighborhoods and assuming they are a large, single population is yet another factor that will cause observed genotypic frequencies to deviate from expected equilibrium frequencies, this phenomenon is known as the Wahlund effect (Falconer 1981, Hederick 1983). More extensive geographical sampling throughout the summer range of *A. sponsa* or comparisons with samples collected in the wintering range of this species could test this hypothesis.

The results of this investigation have managerial implications for migratory avian species that utilize concentrated habitats during the non-breeding season. The genetic diversity of such spiatiotemporal sub-divided populations is probably not an important factor during the breeding season. The genetic integrity of this species is probably much more vulnerable to demographic factors caused by the destruction or degradation of winter habitat. A loss or fragmentation of wintering grounds could be of vital importance to the genetic structuring of such species as fragmentation would destroy the potential for

panmictic mate selection to occur. It would appear that to conserve diversity in *A. sponsa*, efforts must be concentrated on the wintering range of this species; if managed properly there will be minimal consequences of minor habitat degradation and breeding range fragmentation in the summer ranges.

Wood Ducks are highly philopatric (Nichols & Johnson 1990, Baldassarre & Bolen 1994), and this behavior lends itself well to initiate breeding populations outside the normal breeding range of this species. When populations in peripheral areas are originated, individuals migrate along main flyways (Doty & Kruse 1972, 1984), and join individuals from other geographic regions in the winter range. During this migratory and wintering period, mate selection occurs in the presence of individuals from distant breeding regions. This type of mate selection is a mechanism through which high amounts of gene flow can be maintained in peripheral and isolated populations. The behaviors of mate selection are an obviously important component of this managerial strategy; those species in which mate selection does not occur during the winter would be much more prone to the effects of isolation. Hence the reliance of outbreeding through mate selection must be made on a species-specific basis.

The relatively high levels of allozyme variability found in the Hancock captive population are an indicator that the efforts this breeder makes to avoid inbreeding are working fairly well. Attempts are made to regulate inbreeding by replacing hens in the captive flock with hens from other captive flocks in other parts of the country on an annual or semi-annual basis (B. Hancock, pers. comm.). Although the Hancock

population is below that of the ANWR population in all indices, it does not exhibit signs of a highly inbred population. Many captive breeding programs attempt such manipulative gene flow, and it appears to be a reasonable management strategy for Wood Ducks to help maintain adequate levels of genetic diversity.

The ANWR breeding Wood Duck population appears to be declining; whether or not this is actually the case is in question (see other sections of this study). The breeding Wood Ducks at ANWR exhibit high levels of genetic variability in comparison to other waterfowl populations; therefore it is unlikely that reduced genetic variability resulting from founder effects, inbreeding, or population bottlenecks is a major factor responsible for the apparent decline.

Chapter 4

A COMPARISON OF GENETIC DIVERSITY OF THREE WOOD DUCK POPULATIONS USING DNA FINGERPRINTS.

Introduction

DNA fingerprinting is a powerful molecular technique for analyzing individuals based on unique genetic characteristics (Jeffreys *et al.* 1985); because of this accuracy, DNA fingerprinting is useful for identifying individuals within populations and for paternity analyses. This technique utilizes endonuclease recognition sequences in hypervariable minisatellite DNA to create unique (or nearly unique) patterns of banding after exposure to restriction endonucleases, electrophoretic separation, Southern blotting and hybridization with probes of known DNA sequences. Since its inception (Jeffreys *et al.* 1985), DNA-fingerprinting using probes derived from human DNA has been successfully used for forensic purposes (Cohen 1990, Devlin *et al.* 1991), paternity analyses (Quinn *et al.* 1987, Longmire *et al.* 1992), ascertaining the degree of relatedness among groups of individuals (Lynch 1988, Reeve *et al.* 1992), and sex-determinations in sexually monomorphic species (Longmire *et al.* 1993)

Probes derived from minisatellite human genomic sequences have been found useful in analyzing DNA from nearly all taxa; bacteria (Huey & Hall 1989, Ryskov *et al.* 1988), plants (Dallas 1988, Nybom & Schaal 1990, Milgroom *et al.* 1992, Alberte *et al.*

1994), insects (Blanchetot 1991), mammals (Gilbert *et al.* 1990, Reeve *et al.* 1990), and birds (Burke & Bruford 1987, Wetton *et al.* 1987, Kuhnlein *et al.* 1990, Meng *et al.* 1990, Westneat 1990, Piper & Rabenold 1992, Triggs *et al.* 1992). The majority of these studies have dealt with relatedness of individuals within familial lineages or determinations of breeding systems. DNA fingerprinting is not as commonly applied to studies concerned with measurements of genetic variability on a population level (Flint *et al.* 1989, Kuhlein *et al.* 1990, Gilbert *et al.* 1990, Rave *et al.* 1994).

DNA fingerprinting reveals large amounts of individual genetic variability and is potentially a good method for measuring genetic variation in taxa with low levels of allozyme diversity. These genetically depauperate taxa include many rare species or small populations and/or captive populations which may have reduced genetic variance due to inbreeding, bottle-necks or low N_e/N ratios.

Many studies have been conducted using DNA fingerprinting on birds. Birds have nucleated red blood cells, therefore they are an attractive subject for DNA studies; the collection and preparation of nuclear DNA from red blood cells is rather simple and can be obtained in a non-destructive fashion (Longmire *et al.* 1988). Birds also exhibit low levels of allozyme variability making traditional genetic approaches difficult; in comparison to other taxa there is little information on allozyme variability among many avian species. In fact, for many bird species there is more information regarding DNA than allozyme variability.

In this chapter, I investigate levels of genetic variance as measured with DNA fingerprinting in three populations of Wood Ducks. These populations analyzed include the Arrowwood National Wildlife Refuge population, a captive population, and a natural population from the Pacific Flyway.

Materials and Methods

The following populations were sampled: Arrowwood National Wildlife Refuge (ANWR), in eastern-central North Dakota, a captive population maintained by a private aviaculturist in Montana (herein, Hancock), and a native population from western Oregon. This Hancock population was chosen because it is similar to the captive flock used to create the ANWR population; the flock originally used to found the ANWR population is no longer in existence, but the Hancock population is approximately the same size and has been in captivity for a similar length of time. A wild population at the Western Oregon National Wildlife Refuge Complex (herein, Finley), in western-central Oregon, also selected because it is known to have been a small, natural population for a number of years and has had no known genetic inputs from releases of individuals from captive populations.

Wood Ducks were sampled in the field through either the deployment of cannon-nets or trapping individuals in nest-box structures. Blood samples were collected through venous puncture of the brachial vein. The volume of blood obtained from each bird ranged between 250-1000 µl. Blood samples were placed in a microfuge tube containing

400 μl of SET buffer (0.15 M NaCl, 0.05 M Tris-HCl, 0.1 mM EDTA, pH 8.0), and kept cool until the DNA was extracted in the laboratory.

DNA was extracted from whole blood samples using a phenol-chloroform extraction method similar to that described in Maniatas *et al.* (1982). The specific extraction procedures were as follows. Cells were lysed with the addition of 20 μl of 10% SDS; 25 μl of Proteinase-K was added, and the samples were incubated for 12-20 hours at 55°C. This was followed by extractions against 500 μl of water-saturated phenol and two subsequent protein digestions with 25 μl of Proteinase-K, incubated at 37°C for 8-10 hours followed with phenolic extractions. Samples were centrifuged at 12,000 rpm for 10-15 minutes following each phenolic extraction. If samples were still not clear, phenolic extractions were carried out until the supernatant was clear and appeared free of contaminants; final extractions were centrifuged at 12,000 rpm for 30 minutes and the aqueous phase removed. The aqueous phase was then extracted against 500 μl of a phenol:chloroform (1:1), mixture, followed by extraction against an equal volume of $ChCL_3$:IAA. The aqueous phase was removed, and the DNA was precipitated with 0.1X volume of 3 M NaOAC and 2X volumes of 95% EtOH was added. The samples were gently agitated to dissolve the DNA and frozen at -20°C overnight. The following day the DNA was rinsed twice in 70% EtOH and allowed to air dry. The DNA was then hydrated with 300-750 μl of distilled H_2O. This process usually yielded between 3-75 ng of DNA.

Purified DNA samples were stored at -80°C until digested with restriction endonuclease enzymes.

Enzyme digests were carried out according to manufacturer's directions; most digests were carried out in a total volume of 20 µl and incubated at 37 °C for 12-17 hours. Digestion products were then electrophoresed in a 0.8% agarose gel.

Electrophoresis was carried out in submerged horizontal electrophoretic chambers at 25 mA for 12-20 hours. Gels were made with TBE buffer. DNA standards were placed between every five samples in each gel to insure co-migration of equal size fragments across the gel. Phage-Lambda DNA digested with *Hind* III was used as DNA standards in all gels. Wood Duck DNA from a common individual was run as a standard on most gels to insure alignment of fragment patterns between separate gels. Following electrophoresis, gels were stained with Ethidium Bromide (EtBr) and photographed under ultraviolet (UV) illumination; gels were trimmed and the DNA transferred by Southern blotting.

Southern blots were made using Zetabind™ nylon membrane screens. Southern blot transfers were accomplished with a protocol adapted from Maniatis *et al.* (1982), as described in Westneat *et al.* (1988). DNA was denatured by washing twice for 15 minutes in 1.5 M NaCl, 1.5 M NaOH under constant agitation. Gels were then washed twice for 15 minutes in 0.04 M NaOH, 1M NH_4Ac. The DNA was transferred to screens following the methods outlined in Maniatis *et al.* (1982) for a period of 16-18 hours at room

temperature. Southern blots were dried at 80°C under a pressure of 20-27 lbs for 2 hours and then hydrated and washed with 2X SSC for 15 minutes with agitation, followed by a wash for 1 hour at 60°C in 1.0 M Tris (pH 7.5), 0.1X SSC, 0.5 % SDS. Excess SSC was blotted off the screen, and screens were either immediately hybridized or stored at -20°C until used for hybridization.

Hybridization reactions were carried out with the following procedures. The human minisatellite probe, pV47 (Longmire *et al.* 1990), was labeled with the radio-nuclide [^{32}P]dCTP using nick-translation (Rigby *et al.* 1977). The radio-isotope was from New England Nuclear™, 10 mCi/ml. Nick translation was accomplished using a BRL™ Nick-translation Kit following the directions supplied in the kit. Southern blots were pre-hybridized for 5-20 hours at 60°C in 7% SDS, 0.01% BSA, 0.5 M EDTA, 0.5 M Na_2HPO_4. Radio-labeled probe and hybridization buffer were added and hybridization was carried out at 60°C for 18-24 hours. Hybridized filters were washed (2X), in 2X SSC, 0.1% SDS for fifteen minutes at room temperature, followed by a 5 minute wash at 65°C in 0.1X SSC, 0.1% SDS. Autoradiographs were then made using Kodak X-OMAT safety films with exposures at -80°C for times ranging from 12 to 96 hours depending on the specific activity of the filters. Exposures were made until autoradiograms were produced that could be scored.

Autoradiograms were scored by visual examination after close examination of multiple autoradiograms to ensure fragment alignment between different autoradiograms.

Common fragments were identified and used to align samples from different autoradiograms. Several autoradiograms were scored multiple (2-4), times to determine the accuracy of scoring. Fragments were scored as either present or absent, and the resulting matrices of presence-absence data were used to analyze indices of band-sharing.

The mean and variance of the number of fragments for all individuals per population was calculated. Differences in the mean number of fragments per individual between the three populations were evaluated using both a Mann-Whitney U and t-test. Because the original values used to calculate mean number of bands per individual are nominal, the non-parametric Mann-Whitney statistic is probably the most valid test to use (Sokal & Rolhf, 1981). The t-test is most commonly used in the literature, and I use it to achieve consistency with common practices. In cases where there is no discrepancy between the two tests in their levels of significance, t-values are reported. To compare the distribution of different-sized DNA fragments in the three populations, the occurrence of bands was expressed as percent frequencies. Fragment frequencies were expressed as percentages to compensate for unequal sample sizes in the three populations. The frequency of bands was analyzed across the three populations to evaluate patterns of fragment commonness and rarity between the three populations.

The Similarity coefficient (S), of Lynch (1990) was calculated between all pairs of individuals within a population using the following equation:

$$S = 2N_{AB}/(N_A+N_B),$$

where N_{AB} is the number of bands shared between two individuals, and N_A and N_B are the total number of fragments present for individuals A and B. The variance of S was also calculated for each population, and this variance was used to compare variability. The mean number of fragments per individual per population and its associated variance was also calculated. Significance testing of mean S values among the three populations was carried out using a Student's t-test (Sokal & Rolhf 1981).

The expected population homozygosity (EH) was estimated for each population (Lynch 1990) using the following equation:

$$EH = (\Sigma_{k,i}\ p^2_{ki})/ L,$$

where p_{ki} is the frequency of the *i*th allele (fragment), at the *k*th locus (fragment size), and L is the total number of loci (different size fragments) (Lynch 1990). This EH is equivalent to the parameter that Lynch (1990) defines as H; herein I use the abbreviation EH so that it is not confused with the heterozygosity value (H) commonly calculated for allozyme analyses. Under random mating, EH is equivalent to the gametic identity-in-state (I), and in the absence of random mating the two parameters are closely correlated, making EH a reasonable index of the amounts of homozygosity within a population (Lynch 1990).

The interpopulation mean similarity coefficient (S_{ij}), of Lynch (1990) was calculated to assess the degree of differentiation between the populations using the following equation:

$$S_{ij} = 1 + S'_{ij} - (S_i + S_j)/2,$$

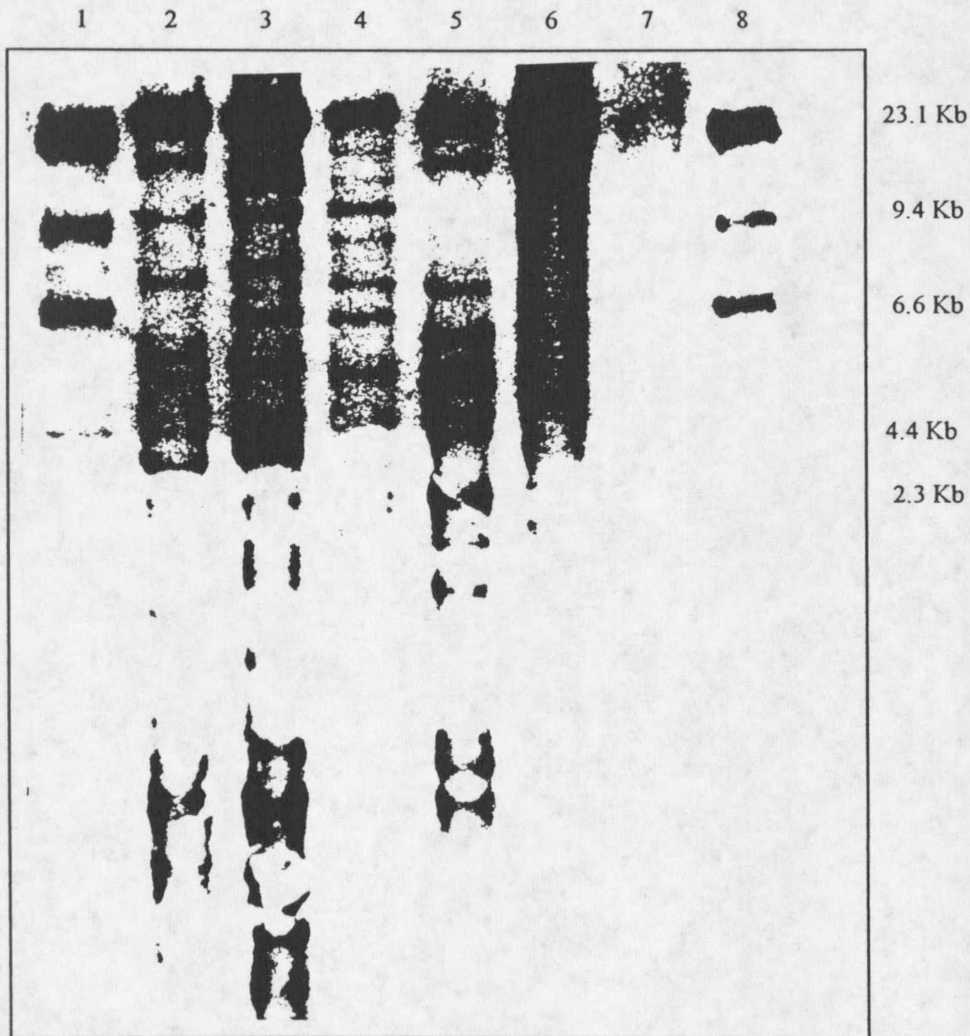
where S_i is the mean similarity of individuals within population *i*, S_j is the mean similarity of individuals within population *j*, and S'_{ij} is the mean similarity between pairs of individuals in population *i* and *j*. The matrix of S_{ij} values was used to create a phenogram of the relationship of the populations under investigation.

Results

An endonuclease-probe screen was conducted to ascertain which enzyme-probe combinations would yield scorable autoradiograms. The enzymes used in this initial screening were: the five-base recognition sequence enzyme *Hinf* I, four-base recognition sequence enzymes *Hae* III, *Sau* IIIA, *Alu* I, and six-base recognition sequence enzymes *Hind* III, *Bam* HI, *Apa* I, *Pst* I, *Bgl* I, *Bgl* II, *Eco*R I, *Pvu* II. The enzymes yielding scorable results using the pV47 probe were *Hae* III, *Hinf* I and *Eco*R I. An example of a typical autoradiogram produced from *Hae* III digests and probed with pV47 can be found in Figure 3.

For the pV47/*Hae* III combination, a total of 58 bands was analyzed for 82 individuals from the three populations; the sizes of these bands ranged from 1.6 Kb to 20 Kb. Over all populations, there were 10.7 (± 1.2) bands per individual (Table 6). For the separate populations, Hancock had the highest mean number of fragments per individual (12.0), followed by ANWR (10.31) and Finley (9.7), respectively. The standard error of these means is 0.72 for Hancock, 1.36 for ANWR and 2.79 for Finley. Statistical

Figure 3. A Typical autoradiogram produced with *HaeIII*/pV47 fingerprints. The lanes are: 1 and 8 - Lambda DNA digested with *Hind*III. Lanes 2 - 4, 6, and 7 are Wood Ducks from Arrowwood National Wildlife Refuge. Lane 5 is a Wood Duck from the Hancock Population.



comparisons reveal that only Hancock and ANWR differ from one-another significantly in mean number of bands per individual ($t = 3.127$, $df = 76$, $\alpha = 0.002$).

Table 6. Genetic diversity values for *Hae* III/pV47 DNA Fingerprints. S is the intrapopulation similarity coefficient, EH is the estimate of homozygosity, MF is the mean number of fragments per individual per population. SE stands for standard error of the mean.

Population	S	SE(S)	n	EH	MF	SE(MF)
ANWR	0.267	0.004	42	0.1341	10.2	1.36
Hancock	0.479	0.014	26	0.2961	12.00	0.72
Finley	0.662	0.005	4	2.937	9.7	2.79

Frequencies of fragments of different size classes for individual populations are given in Figures 4, 5, & 6. The ANWR and Finley populations have more small fragments than does the Hancock population. Because of the low fragment variability and small sample size of the Finley population, it is not included in further analyses of band-size distributions. The cumulative percentages of occurrence of fragments per fragment size class for Hancock and ANWR are given in Table 7 and Figure 7. Although both populations possess fragments throughout the range of fragment size classes, 81% of the fragments in the ANWR population are smaller than 7.0 kB whereas only 58% of the Hancock fragments are less than 7.0 kB in size. The general pattern is that ANWR has more smaller fragments and fewer larger fragments than the Hancock population. The Hancock population has a somewhat even distribution of fragments across all fragment

Figure 4. Frequency (expressed in percentages) of individuals with particular DNA fragments according to fragment size from the Hancock population.

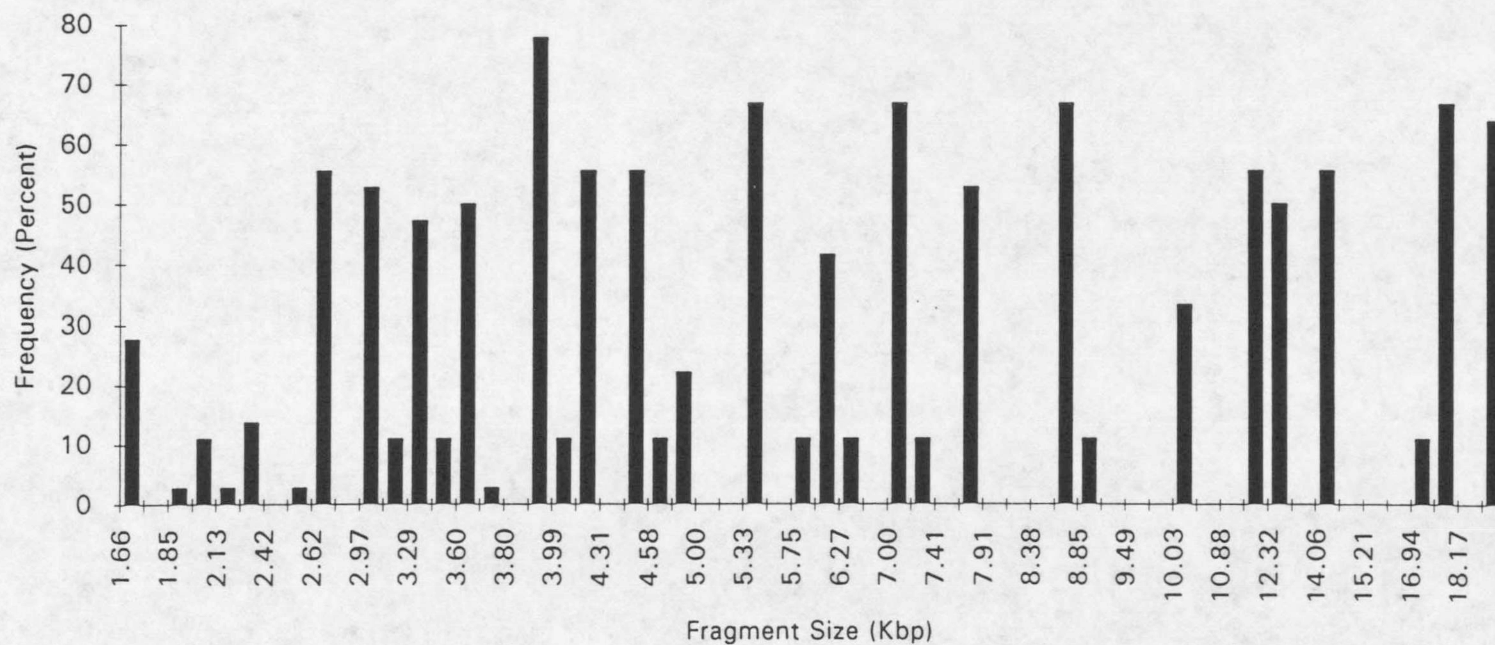


Figure 5. Frequency (expressed in percentages) of individuals with particular DNA fragments according to fragment size from the ANWR population.

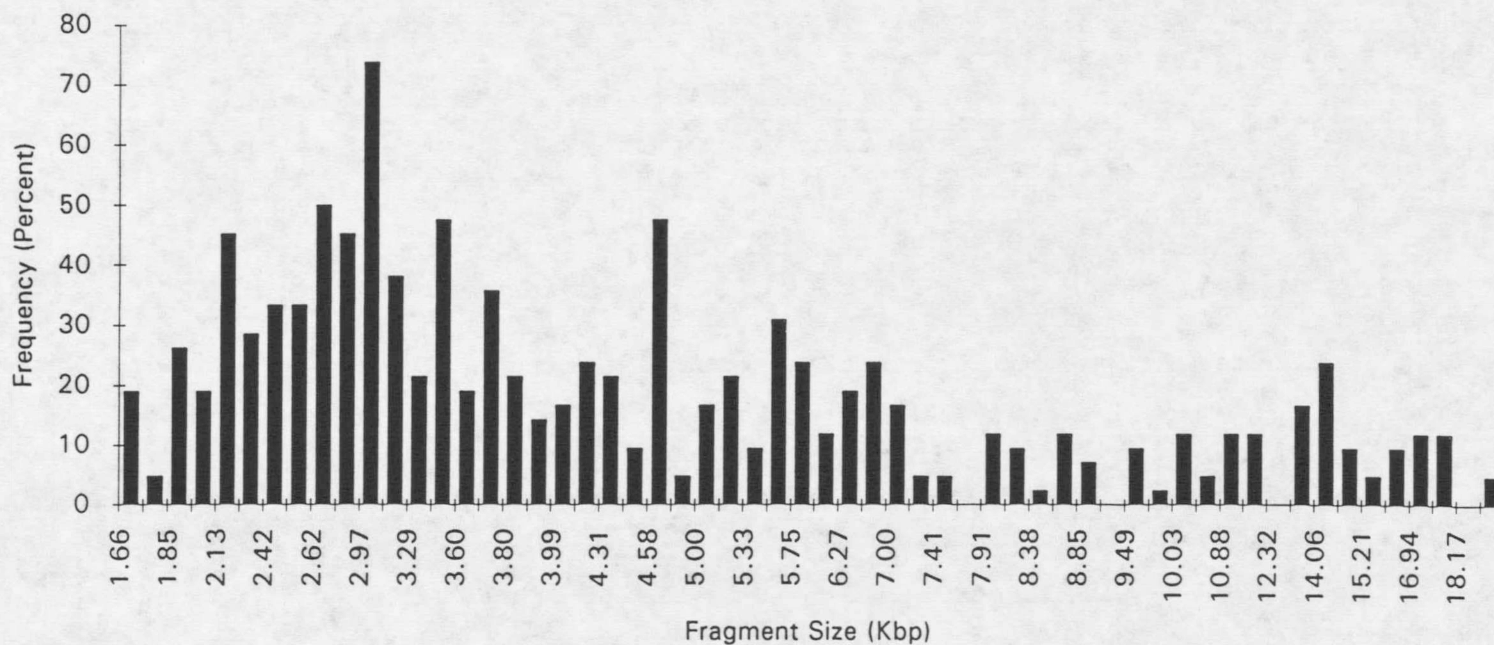


Figure 6. Frequency (expressed in percentages) of individuals with particular DNA fragments according to fragment size from the Finley population.

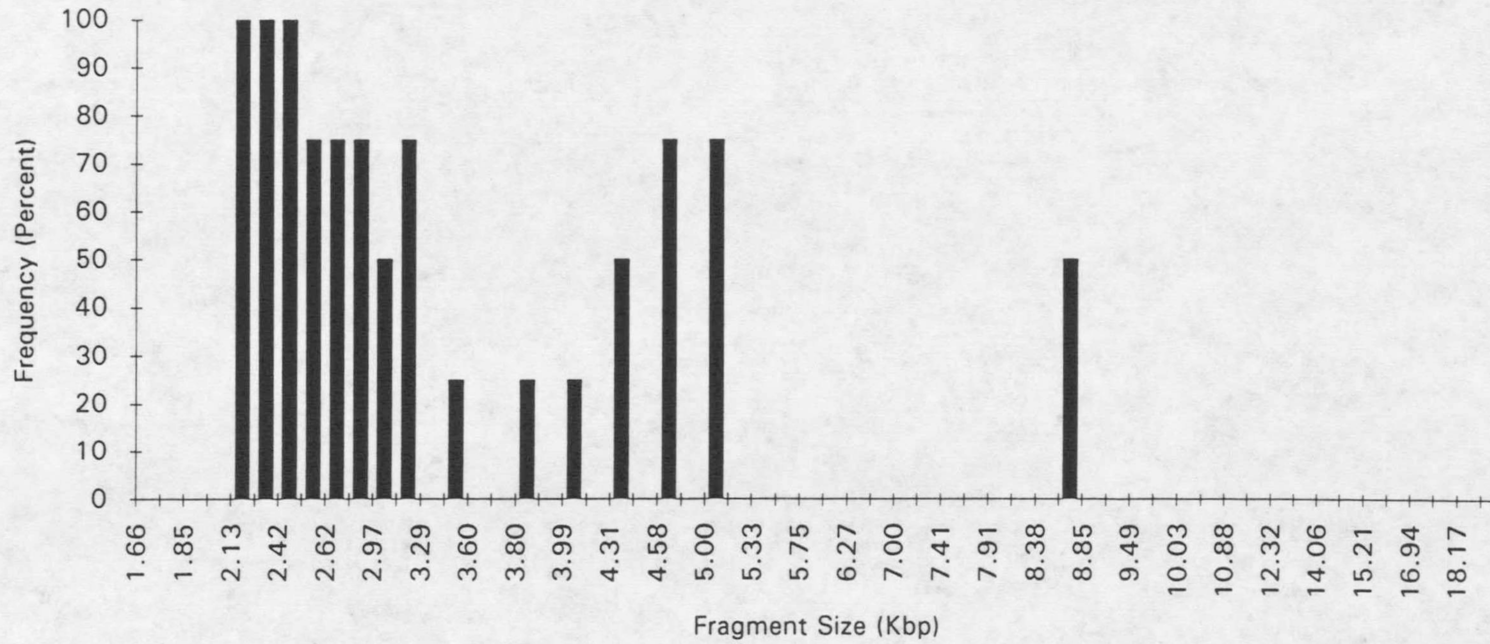
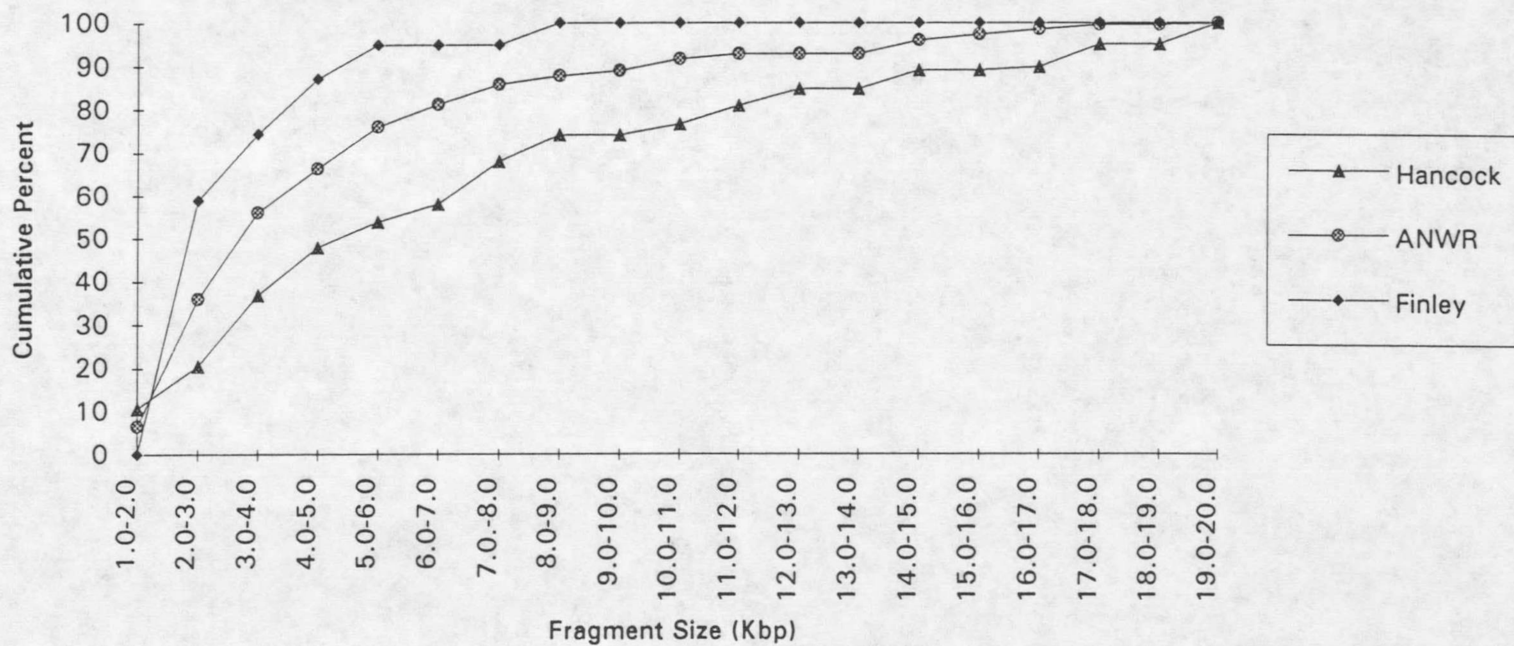


Figure 7. Cumulative percent frequency of DNA fragments as expressed as fragment size for all populations.



size-classes. The Finley population has the highest proportion of small bands of the three populations, and Hancock has the lowest proportion of small-sized fragments.

Table 7. Percentage of fragments by fragment size-class. % Frag., is the percentage of all fragments in that size class. Cum. % is the cumulative percentage of fragments in that size class and smaller size classes.

	Hancock		ANWR		Finley	
Size (kBP)	% Frag.	Cum. %	% Frag.	Cum. %	%Frag.	Cum. %
1.0-2.0	10.7	10.7	6.5	6.5	0.0	0.0
2.0-3.0	9.9	20.6	29.3	35.9	59.0	59.0
3.0-4.0	16.3	36.8	20.3	56.2	15.4	74.4
4.0-5.0	11.1	48.0	10.2	66.4	12.8	87.2
5.0-6.0	6.0	54.0	9.7	76.1	7.7	94.9
6.0-7.0	4.1	58.0	5.2	81.3	0.0	94.9
7.0.-8.0	10.1	68.1	4.5	85.8	0.0	94.9
8.0-9.0	6.0	74.1	2.0	87.8	5.1	100.0
9.0-10.0	0.0	74.1	1.1	88.9	0.0	100.0
10.0-11.0	2.6	76.7	2.7	91.6	0.0	100.0
11.0-12.0	4.3	80.9	1.1	92.8	0.0	100.0
12.0-13.0	3.9	84.8	0.0	92.8	0.0	100.0
13.0-14.0	0.0	84.8	0.0	92.8	0.0	100.0
14.0-15.0	4.3	89.1	3.2	95.9	0.0	100.0
15.0-16.0	0.0	89.1	1.4	97.3	0.0	100.0
16.0-17.0	0.9	89.9	1.1	98.4	0.0	100.0
17.0-18.0	5.1	95.1	1.1	99.5	0.0	100.0
18.0-19.0	0.0	95.1	0.0	99.5	0.0	100.0
19.0-20.0	4.9	100.0	0.5	100.0	0.0	100.0

The results of an analysis of frequency of all fragments (independent of size), is given in Table 8. The ANWR population has more rare bands than the Hancock population. The Hancock population is somewhat bi-modal (Figure 8) in that many bands occur at a frequency of <20%, and a significant proportion of the fragments (40%), occurs in frequencies of 50-70% in the samples analyzed. In the ANWR population, 79.6% of the