GENETIC SUBDIVISION IN SCOTTISH RED DEER AND NORTH AMERICAN WAPITI

by

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ABSTRACT

To better understand the genetic structure of wildlife populations, an isozyme study of Scottish red deer and North American wapiti was conducted. Tissue samples from 943 individuals representing 28 localities were analysed by starch gel electrophoresis. The average amount of variation detected in a random selection of 28 to 34 enzyme loci did not significantly differ from that found in other mammals. There were significant differences between localities in both average heterozygosity and gene frequency. The results suggest that population subdivision characterizes both European red deer and North American wapiti. The polymorphism observed could be used in discriminating both populations and individuals. Nonetheless, the absolute genetic diversity was less than that measured between conspecific deer in Europe and North America. By this electrophoretic measure, red deer and North American wapiti could be considered a single species, (*Cervus elaphus* L.), though not a panmictic population.

Declaration

This thesis has been composed by myself and it has not been submitted in any previous application for a degree. The work reported within was executed by myself, unless otherwise stated.

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CONTENTS

CONTENTS (cont'd)

Chapter 1

INTRODUCTION

1.1 Evolution and classification

Although evolution is recognized as a far reaching paradigm that has increasingly affected all of the life sciences, while taxonomy seems to many a mundane corner of biology obsessed with minute distinctions, the two fields have become inseparable. Fundamental to evolution is the premise that all organisms have a common descent and that there is a systematic hierarchy of relatedness. Following from this is the assumption that features shared by organisms will also be based hierarchically, a reflection of common descent.

Finding characteristics which are fundamental, and thus an accurate reflection of relatedness, is the challenge of classification. It has not always been a case of first time right, as is demonstrated by European red deer and North American wapiti, the subject of this study. Caucasian settlers in North America initially encountered two types of deer, one slightly smaller and the other much larger than the red deer of Europe. The larger animal, too big and dark to be red deer, was called elk after the elk of Scandinavia (Merrill, 1916). More subtle distinctions were either unknown or forgotten by these pioneers (hunting was not a right but a priviledge in the countries from which they came, and many American game laws remain a reaction to this). That size and coat colour were not the best criteria for close common descent must have been obvious to the first systematic observers who found North American moose.

Scandinavian elk and North American moose (Alces alces) are today considered conspecific (Ellerman and Morrison-Scott, 1951; Hall and Kelson, 1959; Whitehead, 1972). It is generally recognized that the elk of North America is the closest relative on that continent to the European red deer (Cervus elaphus L.). Whether red deer and elk are one species or two is still debated, and it is among the questions which this study attempts to address. Unfortunately, the common name "elk" has survived in North America, although it is a misnomer which continues to cause confusion. Many scientists prefer the Shawnee name wapiti (light rump) to avoid this confusion with the Scandinavian animal. That practice is adopted in this thesis, and moose will refer to all Alces alces, abandoning "elk" altogether.

In postulating natural selection, Darwin and Wallace not only provided a mechanism for evolution, but a critical link with taxonomy: individuals which were better adapted to their particular environment would have more offspring which would carry those adaptive traits. The inheritance of locally adaptive characters causally related common descent to the common structures on which taxonomy was based.

Corollaries to natural selection, such as Bergmann's Rule, not only helped to explain misadventures in taxonomy, but also affected the classification of newly-discovered organisms. Bergmann's Rule (Mayr, 1963, p.319-320) maintains that due to increased metabolic efficiency, animals in colder climates will have larger body sizes, assuming that they can get the resources necessary to maintain them. Thus wapiti are not bigger because they come from America, but because they came to North America via the Arctic through the Pleistocene. Moreover, fossil evidence indicates that Pleistocene red deer (Walvius, 1961) and wapiti (Guthrie, 1966) were both larger than

Recent forms. Differences in available cover and food quality explain why most wapiti have shrunk less than red deer since the Ice Age. There is one wapiti subspecies, the "Tule elk," living in poor, open habitat which shows a relative size reduction similar to that of Scottish red deer (McCullough, 1969).

The example elaborated above illustrates what Ehrlich (1965) called the "comfortable circularity" which pervaded biology for the century following the publication of Darwin's (1859) work. Evolution was invented to explain the taxonomic order found in nature: the taxonomic system in turn was used as positive proof of evolution. In recent years, both numerical taxonomy and cladistics have developed to divorce taxonomy of its evolutionary bent in evaluating phenotypic traits.

Darwin's work did not explain how adaptive traits passed from successful parents to their offspring. Within a decade of the publication of On the Origin of Species, the engineering professor at Edinburgh pointed out that blending inheritance, the genetics dogma of the day, was inconsistent with natural selection (Dunn, 1965). If offspring averaged the characteristics of their parents, then adaptive advantages would be diluted by half in each generation and soon be blended into oblivion. Darwin himself had to resort to the inheritance of acquired characters to explain the variation on which selection could act.

It was not until Weismann demonstrated the particulate nature of genetic material, and the rediscovery of Mendel's experiments showing the segregation of that material in each generation, that there was a genetic system consistent with the variation implicit in natural selection. The units of genetic material, genes, are located at

particular sites, or loci, on chromosomes. The different forms of the gene are defined as alleles, and the particular combination of alleles in an organism comprise its genotype for each locus. The expression of the genotype, as affected by the environment, manifests changes in the phenotypes of an organism. If the relationship between the frequency of different alleles and phenotypes can be established, the differences between individuals, populations, species, etc. could be quantified. Thus genetics became a partner with other disciplines in the study of common descent and this Huxley (1942) called the Modern Synthesis.

The practical problems of measuring genetic differences in natural populations remained formidable, as Lewontin (1974, p.96) points out: "For phenotypes of evolutionary interest, like size, shape, metabolic rates and probabilities of survival and reproduction, the average effects of gene substitution are small compared with the variation from environmental fluctuation. The counting of genotypes in a population, however, requires that the differences in phenotype produced by allelic substitutions be large enough to allow unambiguous classification into genetic classes." Among the obstacles were the masking of one allele due to dominanace, as well as determining the allelic inheritance when many genes contributed to the phenotype.

Some of these problems were overcome by breakthroughs in biochemical genetics. A critical discovery was the one-to-one relationship between the nucleotides which make up structural genes and the amino acid sequence which codes for enzymes and other proteins. A change in the amino acid sequence could therefore be generally ascribed to an allelic substitution. Moreover, as some of the amino acids have an electrostatic charge, many amino acid

substitutions result in a change in the net charge of the enzyme or protein for which they code. When net changes in the charge occur, they can be used to separate the enzymes or proteins and thus identify different alleles of the same gene. The technique of separating proteins by observing their migration rates in an electric field is called gel electrophoresis (Markert and Møller, 1959).

Electrophoresis is the method used for assessing common descent in this study, and it is detailed further in later sections (1.3, 2.2). There are related advantages and disadvantages in using this technique on red deer and wapiti. The primary benefit is clarity: there is much less ambiguity in relating phenotypes determined electrophoretically to genotypes and ultimately to allele frequencies than by most other methods (for this reason, electrophoretic phenotypes are commonly referred to as genotypes in the scientific literature). For example, in the case of perfect dominanace, one allele would be masked phenotypically, but both forms of the gene are visible on an electrophoretic gel. When two forms of the same enzyme are observed in the same sample, they are the products of different alleles and the individual is heterozygous at that locus. A logistical advantage is that enzymes active in somatic cells can be analysed from just a small tissue sample, and the whole animal is not required for comparison.

The cost in making the point of comparison closer to the actual genetic material, DNA, is that it takes a step away from the structures on which selection likely operates, many of which are probably the result of multi-genic effects. Furthermore, the precise function of alternate alleles for many of these enzymes and proteins is not known, nor has it been proved that they afford any selective

benefit. For these reasons it is necessary to survey a wide range of enzyme and/or protein loci, regardless of the variation observed, to make an objective comparison between organisms. This point is made emphatically by Lewontin (1974, p.96), one of the pioneers in applying electrophoresis to natural populations, though it has been ignored in many subsequent studies.

These points can be illustrated by assuming, simplistically, that there is a single locus which determines the number of legs an animal has. Red deer and wapiti are monomorphic for leg number, i.e. they show no, or nearly no, variation. One could probably count legs for several millennia and see no significant difference between red deer and wapiti. This is the premise by which wildlife biologists sometimes use legs to count deer when they are tightly grouped. But a leg count could tell something quite fundamental about the relationship between deer and dolphins. Although deer have an unvarying number of legs, as do dolphins, an analysis of the two groups together, in evaluation of mammals, would show overall polymorphism for this trait. Here the analogy breaks down, and at the same time illustrates why electrophoresis was grasped by evolutionary biologists as a powerful tool. Since dolphins do not have legs, the relationship between dolphins and deer is obscured at this phenotypic level; electrophoresis usually records fixed differences regardless of their phenotypic expression. However, to prove closer common descent between deer and dolphins, than deer and fish, one would have to look at more of the genome than the leg locus.

Despite the drawbacks of electrophoresis or any other method, classification is essential not only in academic studies of evolution, but in the management of wild populations. Conservation, like

classification, begins with the description of unique characters, and only if animals can be adequately differentiated can strategies sensitive to local conditions be developed. The remaining introductory sections describe the study animals in more detail, the population genetic principles which were applied, and the previous electrophoretic research on deer, particularly red deer and wapiti.

1.2 European red deer and North American wapiti

Red deer and wapiti are members of a deer genus which extended across the the northern temperate latitudes worldwide at the end of the last glaciation. The source of this radiation was probably Asia, and it has two major branches (Lydekker, 1898; Cameron, 1923; Murie, 1951). The eastern extreme is represented by wapiti in North America, the western by red deer in Ireland, Britain and Spain. There are still eleven recognized subspecies in Asia (Groves and Grubb, in press).

Wapiti, when originally named by Erxleben in 1777, were considered a subspecies, Cervus elaphus canadensis. In 1780, however, Borowsky decided that the North American animals were a unique species, and dropped elaphus (Hall and Kelson, 1959). Through the first half of this century, Cervus canadensis was generally recognized (Murie, 1951), though there were some detractors in Europe (Ellerman and Morrison-Scott, 1951). The recent trend among zoologists and wildlife managers has been to eliminate this distinction and consider red deer and elk conspecific (McCullough, 1969; Bryant and Maser, 1982). The primary reason for this relegation has been documented evidence of hybridization, both in captivity (Whitehead, 1951) and in the wild where animals have been introduced (Murie, 1966; Caughley,

1971). This follows Mayr's definition (1963, p.19) of a species as "groups of actually or potentially interbreeding individuals which are reproductively isolated from other such individuals."

Though not reproductively isolated, there are obvious differences between red deer and North American wapiti (Walker et al., 1975). Wapiti are significantly larger, have a more well developed rump patch and a relatively smaller tail. Wapiti bulls have more branching terminal antler tines and a higher pitched mating "bugle." Red deer, on the other hand, have less colour contrast in their reddish coat, and the stags have a characteristic "roar" during the rut.

Hunting and habitat alteration have caused the present highly dissected distribution of red deer and wapiti (Figure 1). Though found in many rural and reforested areas of Britain, red deer are most abundant in Scotland, numbering about a quarter of a million (Red Deer Commission, 1979, p.10). Wapiti were once distributed across North America, but are now common only in the mountainous regions of the western part of the continent, where they number about half a million - an estimated 5% of their population size before the arrival of European man (Bryant and Maser, 1982, p.1). Their current distribution, as with with Scottish red deer, is not necessarily evidence of particular adaptation to rugged habitats, but rather a reflection of the areas where such large, gregarious animals are tolerated.

There are at present four recognized North American wapiti subspecies and at least seven subspecies of European red deer. These subspecies have been differentiated on the basis of pelage differences and skeletal measurements (Murie, 1951; Ahlen, 1965). More recent skull comparisons, however, suggest that the division between

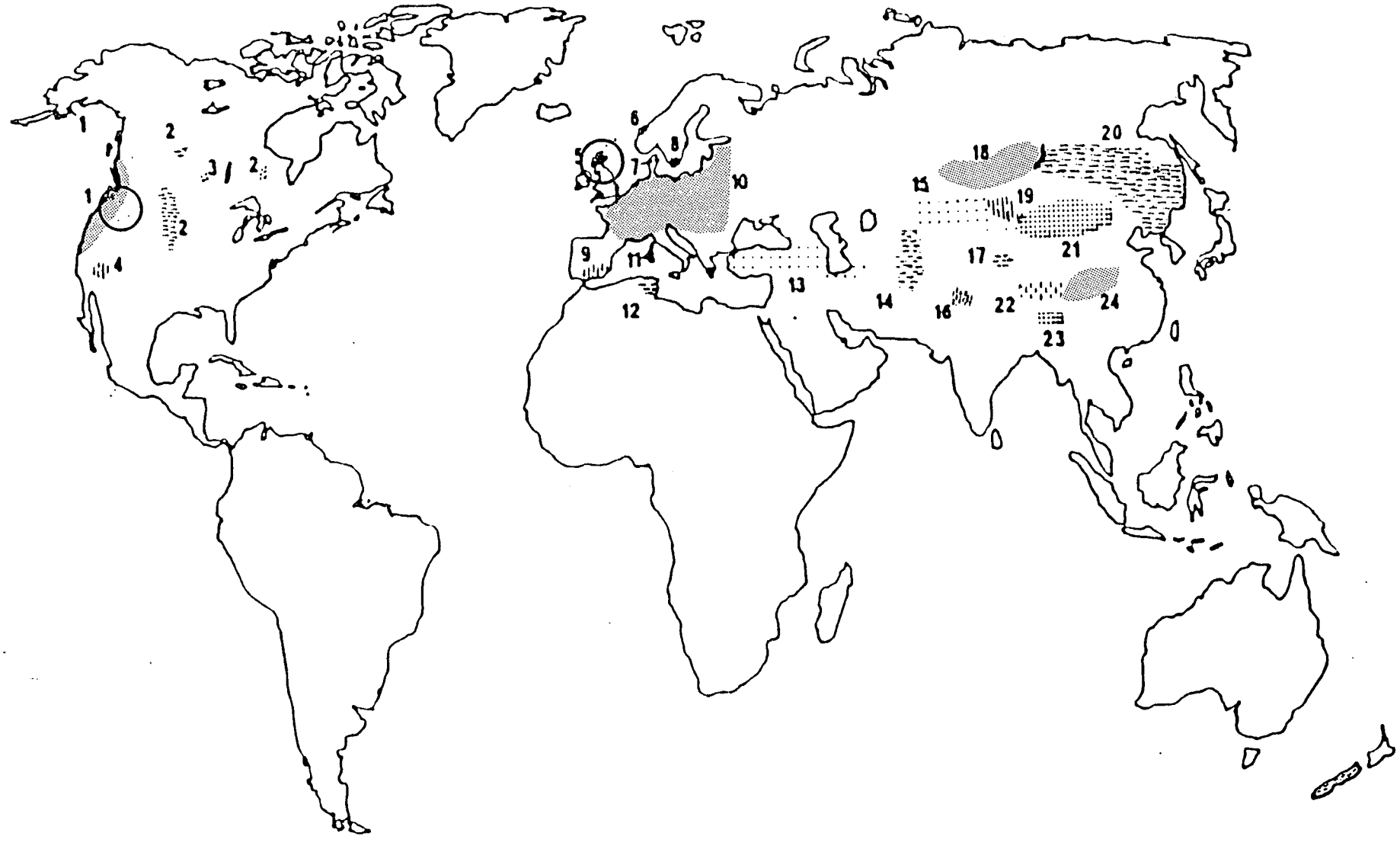


Figure 1. World distribution of red deer and wapiti. Locations of presumed subspecies are numbered. Study areas where samples were collected are circled.

subspecies is not clear-cut, either within red deer (Lowe and Gardiner, 1974) or within wapiti (Shonewald-Cox and Bayless, *in prep.*).

Though red deer and wapiti in particular areas have been designated as rare, the genetic characteristics of these animals have usually not been investigated. The great expansion in captive breeding of deer (Yerex, 1982) has stimulated recent interest in their genetics, though progeny tests (Lincoln *et al.*, 1973, 1976; Lincoln and Fletcher, 1977) are still uncommon and population genetics principles have not generally been used in their management.

1.3 Applying population genetics

Ever since Mendel's experiments were rediscovered, and infused with mathematics in the 1930's, the knowledge of how genes act in populations has grown (Provine, 1971). Initial observations were on domestic and laboratory animals, but the advent of biochemical methods, particularly electrophoresis, made it possible to measure genetic differences in natural populations (Harris, 1966; Lewontin and Hubby, 1966).

Studies in population genetics often begin by describing the genotypes present and counting the numbers of each. In the case of electrophoresis, genotypes are alternate forms of enzymes or other proteins which migrate at different rates depending on their charge, size and shape. The different forms of an enzyme are called isozymes (Markert and Møller, 1959) or allozymes (Prakash *et al.*, 1969) when specifying that the alternate forms are the product of different alleles. They can be visualized as bands on a gel of starch, agarose, acrylamide or some other neutral medium when stained with the

appropriate co-factor to effect the enzyme reaction. Table 1 lists the genotypes and their proportions for the enzyme glucose phosphate isomerase (GPI-1), as sampled in one Scottish red deer population.

Banding patterns observed	100/100	100/160	160/160	Total
Genotype numbers	35	5	1	41
Frequency	0.854	0.122	0.024	1
Notation	P	H	Q	1

Table 1. Genotype frequencies for GPI-1, Rhum population, 1979.

The genotype frequencies, however, describe only the static characteristics of one population at one time. Because deer are diploid, the genotypes are split during meiosis and each parent contributes half of the genes to the genotype of their calf. Since the changes in a population are transmitted by genes and not genotypes, it is the gene frequencies which must be calculated in comparing populations. This is done in Table 2 for the same enzyme and population.

	100/100	100/160	160/160	Total
Number of individuals	35	5	1	41
No. of GPI (100) alleles	70	5		75
No. of GPI (160) alleles		5	2	7
				82

Gene frequency of GPI (100) = 75/82 = 0.9146 = p
Gene frequency of GPI (160) = 7/82 = 0.0854 = q
1.00

Table 2. Gene frequencies calculated from genotypes for Rhum deer.

From the two previous tables, the general relationship between gene and genotype frequencies is evident: $p = P + 1/2\ H$ and $q = Q + 1/2\ H$. Mutation, migration, selection, small population size, gene linkage, non-random mating, and overlapping generations can all act to change gene frequencies of sexually reproducing animals such as

deer. In the absence of these factors, however, the frequencies will remain constant and the genotypes of the next generation can be predicted by the gene frequencies of the parental population: $(p + q)^2 = p^2 + 2pq + q^2$. It is the segregation of alleles every generation which preserves variation, as mentioned earlier. This result was proved independently in 1908 by T. Hardy in England and W. Weinberg in Germany and the binomial distribution of alleles is at the foundation of population genetics.

The assumptions of the Hardy-Weinberg Law, when violated, are the elements of differentiation between populations. For example, when there is not one large random-mating population but many smaller ones (as is the probable case with the red deer/wapiti group as shown in Figure 1), then the genotypes sampled across populations at the same time should differ in a predictable way.

When population size is restricted, the gene frequencies are no longer stable because the gametes of the parents are not necessarily a random sample of the population at large. Even in the absence of local selection pressures, the gene frequencies of the smaller populations, measured separately, may not mirror the gene frequency of the larger population. For instance, the gene frequencies given in Table 2 for glucose phosphate isomerase on the Isle of Rhum were GPI-1 (100) = 0.9 and GPI-1 (160) = 0.1. Suppose the Rhum deer were not a single panmictic population (and there is some evidence to support this suggestion, McDougall and Lowe, 1968), but several much smaller breeding units. By chance some of the subpopulations could be composed entirely of GPI-1 (100) homozygotes; others would have a higher frequency of heterozygotes and in these the allele frequency for GPI-1 (100) would be reduced. Population genetics theory makes

several predictions about both gene (or allele) frequencies and genotype frequencies when a large randomly-mating population becomes subdivided.

First, the average gene frequency of the subpopulations equals that of the original undivided population. On Rhum, p = 0.9 both in the original population (p_o) and the average of the subpopulations (p). Within each subpopulation, the gene frequencies change due to sampling differences, and therefore the gene frequencies of the subpopulations diverge. However, there is a limit to how far they can diverge as alleles either become fixed (the entire subpopulation is homozygous, p = 1) or lost (p = 0). Given the original gene frequencies on Rhum (p_o = 0.9 and q_o =0.1), it would be expected that in 90% of the subpopulations GPI-1 (100) would become fixed; in 10% of the subpopulations GPI-1 (100) would be lost. In this two allele system, the proportions for GPI-1 (160) are simply the inverse of those for GPI-1 (100).

As the gene frequency approaches fixation or loss in each subpopulation, the proportion of homozygous genotypes increases to the detriment of heterozygotes. In the extreme case, each deer would be homozygous for one allele and there would be no heterozygous animals; if all of the subpopulations were at this limit, each would have homozygotes for one allele, though not the same one. As a consequence of this tendency towards homozygosity in small populations, when all subpopulations are pooled, there is a deficiency of heterozygotes from what would be expected given the overall population gene frequency (On Rhum, p_o = p = 0.9 therefore $2p_oq_o$ =0.18). It is the deficiency of observed heterozygotes from expected binomial proportions which can be used to infer whether populations have differentiated due to genetic

drift.

The assumptions for this model of genetic subdivison - particularly constant size for each subpopulation and no migration between them - are biologically unrealistic. Researchers on Rhum have long noted that a stag often does not rut near his birthplace on the island (Lincoln, Albon, pers. com.) However, when population subdivision is substantial, a significant deficiency in heterozygotes is still observable. This is shown below by pooling red deer and wapiti populations at the SOD-1 locus.

	Observed Genotype Frequency			Gene Frequency	
SOD-1	100/100	100/125	225/225	100	225
	P	H	Q	p	q
Red deer	0.04	0.13	0.83	0.10	0.90
Wapiti	0.82	0.10	0.07	0.88	0.12
Pooled	0.43	0.12	0.45	0.49	0.51

	Expected Genotype Frequency		
	p^2	2pq	q^2
Red deer	0.011	0.188	0.801
Wapiti	0.774	0.211	0.014
Pooled	0.24	0.50	0.26

Table 3. Gene and genotype frequencies for red deer and wapiti populations calculated independently and combined. The greatest deficiency in heterozygotes is in the pooled population (Observed 12%, Expected 50%).

In all of the examples above, the observed frequency of heterozygotes is less than the predicted Hardy-Weinberg proportions. This is due to the fact that the red deer and wapiti samples also come from several populations. By far the largest deficiency in heterozygotes, however, is in the red deer and wapiti together. The significance of this difference between observed and expected genotype

frequencies can be simply tested by chi-square, as is done in Chapters 4 and 6 for all of the enzyme loci analysed.

The reduction in heterozygotes is a function of how much the gene frequencies in each population have drifted apart since divergence, which in turn is affected by the degree of inbreeding within each population. Inbreeding, generally, is the number of ancestors that two individuals have in common.

The inevitablility of common ancestry can be deduced, again using the Scottish deer population. Each calf has two parents, four grandparents, etc., so that the number of direct ancestors is $(2)^t$ where t is the number of generations that are being considered. Going back twenty generations, a calf is directly descended from 1,048,576 deer, about four times the current Scottish red deer population, and probably even more than that of the population twenty generations ago (between 100 and 120 years) when sheep were more prevalent in the Highlands (McConnochie, 1923). Thus, there were not enough ancestors to go around for each calf born now not to have some in common.

As a population becomes more genetically isolated from the red deer population at large, the rate of inbreeding within the smaller population increases concomitantly. In population genetics terms, the amount of inbreeding (known as the inbreeding coefficient when comparing individuals or the inbreeding index when comparing populations, and generally symbolized by F) is the probability that two genes at any locus are identical by descent. Whereas in the population at large the heterozygote frequency is 2pq, in the smaller population it is 2pq(1-F) where 1-F measures the reduction in heterozygosity due to inbreeding.

By comparing the gene frequencies of several smaller populations with the average gene frequency for them taken together, the degree of inbreeding - and hence the amount of genetic drift at a particular locus - can be determined. This is done in Table 4 with three Scottish populations for the SOD-1 locus.

$$1 - F = \frac{\overline{2pq} \text{ of each smaller population } (H_s)}{2p_o q_o \text{ in the population at large } (H_t)}$$

	Caithness	Loch Laggan	Strathmashie	
p	0.86	0.66	0.93	0.816 $\bar{p}$
2pq	0.24	0.45	0.13	0.273 H_s

$$\overline{2pq} = H_s = (0.24 + 0.45 + 0.13) / 3 = 0.273$$

$$2p_o q_o = H_t = 2\ (0.816)\ (0.184) = 0.299$$

$$1 - F = 0.273 / 0.299 = 0.913$$

$$F = 0.087$$

Table 4. Calculation of the inbreeding index (F) for three populations of Scottish red deer at the SOD-1 locus, p is the average gene frequency for the three populations (q = 1 - p). H_s is the average of the heterozygosities for the individual populations and H_t is the total heterozygosity.

The inbreeding index calculated in Table 4 provides a measure of the divergence in gene frequency between these three red deer populations at this particular locus. Although originally intended to analyse inbreeding, Sewell Wright (1940, 1965, 1978) extended the concept and devised a series of F-statistics to measure the genetic divergence between natural populations of both plants and animals.

In analysing many genetic loci, the divergence is measured by comparing the average heterozygosity of overall population (H_t) with the average of the heterozygosities of subpopulations which comprise it (H_s) to get an overall fixation index (F_{st}), also called the standardized gene frequency variation among subpopulations (Wright, 1943). It can be derived directly from the the previous inbreeding

coefficient, substituting F_{st} for F, and noting that H_s and H_t are calculated from more than one locus:

$$1 - Fst = Hs / Ht$$
$$Ht\ (1 - Fst) = Hs$$
$$Ht - Ht(Fst) = Hs$$
$$-Ht(Fst) = Hs - Ht$$
$$Fst = (Ht - Hs) / Ht$$

In Table 5, two more polymorphic enzyme loci are added to the data for the three previous Scottish deer populations, and the fixation index is calculated using the formula above: $F_{st} = (H_t - H_s) / H_t$

	CAITHNESS	LOCHLAGGAN	STRATHMASHIE	H_t	
IDH-2	0.525	0.868	0.577	0.4054	
GPI-1	1.00	0.882	0.962	0.0938	
SOD-1	0.857	0.658	0.929	0.2757	
				0.2838	H_t
H_s	0.2480	0.2958	0.2311	0.2583	H_s

$$F_{st} = 0.2838 - 0.2583) / 0.2838 = 0.090$$

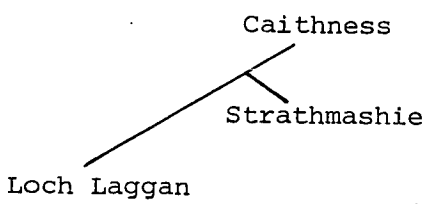
Table 5. Relative genetic divergence of three Scottish red deer populations calculated using F_{st} for three polymorphic loci.

The fixation index above provides a measure of the difference in the gene frequencies for these loci that is due to the breeding isolation of these populations. In other words, 9% of the genetic variation described is due to differences between populations, and the remainder is due to differences between individuals within populations. Adding loci which are monomorphic for the same allele in all populations will not alter the fixation index, as they do not contribute to the between-population variance.

The inclusion of monomorphic loci would, however, decrease the absolute genetic diversity (Dm) among populations, as defined by Nei

(1975): $Dm = n (H_t - H_s) / (n - 1)$ where n is the is the number of subpopulations. This is because the monomorphic loci reduce both the total and subpopulation heterozygosity. Just as with the leg locus mentioned earlier, they are necessarily included to make objective comparisons with other organisms.

Finally, gene frequencies can be used to calculate a measure of genetic distance and thus indicate which populations have the closest common descent, i.e. those populations which have diverged least for the loci observed. Noting that most estimates of genetic distance are related in some way to the fixation index (F_{st}), Hartl (1982, p.168) suggests the formula $F_{st} = 2d^2$ as a first approximation in constructing trees of relationship between populations. This is done in Table 6 for the same three Scottish deer populations. The distance (d) between all populations is calculated; those with the shortest distance are then paired; their gene frequencies are averaged, and the process is repeated.



	$F_{st} = 2d^2$	d
Loch Laggan / Caithness	0.095	0.213
Loch Laggan / Strathmashie	0.094	0.217
Caithness / Strathmashie	0.006	0.057
Caithness and Strathmasie/ Loch Laggan	0.093	0.216

Table 6. Genetic distances between three red deer locations in Scotland based on isozyme variation at GPI-1, IDH-2 and SOD-1.

The branching between the populations listed is readily confirmed by returning to the gene frequencies listed in Table 5. At each locus Loch Loggan has the most different frequency, and in the tree

constructed it is the most distant limb. The tree, though providing a visual image of the distance between these populations, is inadequate in two ways. Because confidence intervals or standard errors are not included, there is no means by which to judge the reliability of the branch lengths. Also, it lacks an absolute scale. Regardless of how accurately the distances reflect the relationship between these deer populations, it is a relative measurement which could not be used for comparison with other animals, except those measured under the same conditions at the same loci. To extend the tree analogy, there is no way of knowing whether this is a dwarf alpine fir of the mountain ridge (Abies lasiocarpa) or a great Douglas fir in the valley (Pseudotsuga menzezeii). For this reason, Felsenstein (1981) refers to such trees as "unrooted." To extrapolate from one tree to another, the measurement of genetic distance must consider both monomorphic and polymorphic loci.

In analysing the genetic variation within a species, the absolute genetic divergence can often be quite small - not surprisingly, the morphological similarity by which they were originally grouped is certainly genetic - yet the relative divergence can be significant, even over small geographic areas (Selander et al., 1969; Manlove et al., 1975). The application of these population genetics principles depends ultimately on the number of different genotypes that can be described and quantified. The tissue and/or blood sample from each deer, analysed by electrophoresis, potentially provides information on many genetic loci.

1.4 Electrophoretic studies of deer.

Since the first reported research more than twenty years ago

(Lowe and McDougall, 1961; Gahne and Rendel, 1961), there have been more than sixty published studies of deer using electrophoresis or related biochemical methods. Although conventional electrophoresis detects genetic differences which result in net changes in ionic charge, and perhaps gross alteration in size and shape or protein molecules (Johnson, 1977), some allelic variation has been observed in most studies where more than a few individuals within a species have been tested. It should be added, moreover, that even the most intensive electrophoretic surveys analyse only a small fraction of the genome of any organism.

As there has been no published summary of the electrophoresis literature for deer, a systematic review was conducted in the course of this study, and it is included as Appendix 1. Here the findings are summarized, and only the studies of the red deer/wapiti group are detailed. The proteins analysed in deer have been primarily two types: those which are components of blood (i.e. haemoglobin, transferrin albumin, etc.) and enzymes which are concentrated in body tissues (most commonly striated muscle, liver and kidney).

Haemoglobin polymorphism has been found in most deer species where it has been investigated, and white-tailed deer (Odocoileus virginianus) have been most thoroughly studied because the blood of this species has a sickling trait similar to that found in humans (Kitchen et al., 1964, 1966, 1967; Huisman et al., 1968). Several of the more recent haemoglobin studies have been for forensic purposes, comparing the blood of captive deer to detect species differences (Dilworth and McKenzie, 1970; Bunch et al., 1976; Butcher and Hawkey, 1977). Transferrin variation has also been found in several species, and more alleles have been described for reindeer (Rangifer terandus)

transferrin than for any other locus (Braend, 1964b; Zhurkevich and Fomicheva, 1976). The deer species in which blood proteins have been studied and the number of alleles detected are listed in the table below.

SPECIES	BLOOD PROTEINS			
	Albumin	Haemoglobin	Transferrin	Myoglobin
Cervus elaphus	2	2	4	1
C. duvoceli	-	2	-	-
C. timorensis	-	2	-	-
Elaphurus davidanus	1	-	1	-
Dama dama	1	1	1	-
Axis axis	-	2	-	-
Rangifer terandus	2	1	10	-
Alces alces	1	1	2	1
Capriolus capriolus	1	1	1	-
Odocoileus virginianus	1	7	7	1
O. hemionus	1	2	-	-
Ozotocerus bezorctus	-	-	6	-
Hydropotes inermis	-	2	-	-
Muntjac reevesii	1	2	1	-
Polymorphic species	22%	67%	56%	0

Table 7. Number of alleles for four commonly studied blood proteins and, below the line, the percentage of species which are polymorphic.

Electrophoretic research on deer enzyme proteins began later (Manlove *et al.*, 1975) than blood protein studies, but the work has greatly expanded in recent years. There are still only six deer species where extensive enzyme studies have been conducted and these are all native to the Northern Hemisphere. The lowest level of enzyme variation has been recorded in fallow deer (*Dama dama*) (Pemberton, 1983) and the highest in white-tailed deer (Smith *et al.*, *in press*), both deer species of intermediate size.

Some enzyme systems (GPI-1, IDH-2, MDH-2, MPI, 6PGD, PGM-1) appear to have higher percentages of polymorphism across deer species. This non-random distribution of polymorphic loci has also been observed by O'Brien *et al.* (1980) in a comparison of cats, mice and

humans. There is as yet no single satisfactory explanation why some enzymes harbour more detectable allelic variation. The observation nonetheless provides a starting point if genetic markers are sought in an unstudied species or population.

There has been only one published electrophoretic comparison between red deer and wapiti (Johnson, 1968). Though he found no general blood protein differences between 64 wapiti and one red deer, Johnson concluded: "A word of caution, however, is in order. The family Cervidae have been found to contain simple proteins by the paper technic (sic). More detailed technics have been found to be confusing because of variability of minor protein fractions. Analysis of specific proteins may show other distinctions."

Studying blood samples from Scottish red deer, McDougall and Lowe (1968) were the first to find differences in transferrin gene frequencies from different populations. McDougall and Stewart (1976) have also found polymorphism in the whey proteins of red deer milk. The transferrin variation was subsequently confirmed for red deer in Germany (Bergmann, 1976) and Scandinavia (Gyllensten et al., 1980). Cameron and Vyse (1978), however, found no transferrin variation in the Yellowstone wapiti population; screening 24 protein loci, they found polymorphism at only one locus on isocitrate dehydrogenase (IDH-2). In a recent study of single populations of red deer and wapiti, Baccus et al. (1983) found polymorphism at MPI as well as IDH-2.

Despite the plethora of electrophoretic research on deer found in a wide range of journals, there have been relatively few studies which have used isozymes to examine the structure of populations, particularly from a management perspective. The most extensive population studies of deer, using both blood proteins and tissue

enzymes, have been done on white-tailed deer in the southwestern United States (Manlove et al., 1975; Manlove et al., 1976; Johns et al., 1977; Ramsey et al., 1979; Chesser et al., 1982.; Smith et al., in press), and on moose in Scandinavia (Ryman et al, 1977; Reuterwall, 1980; Ryman et al, 1980). The work of these two groups, who have at times collaborated, is of particular relevance to the current study, especially for comparative purposes.

The initial purpose of the present project was to survey specific protein variation in both red deer and wapiti. Tissue enzymes were chosen over blood proteins because they have been less tested previously, and a wide range of loci could be studied that were an unbiased sample with regard to function or variation. From a practical standpoint tissue samples are readily available from culled deer, and can be collected and stored in remote areas.

The primary questions which I have tried to answer in this biochemical comparison of red deer and wapiti are threefold. 1) Is there significant genetic variation in the tissue enzymes of these animals? 2) If so, does this variation differentiate deer from different geographic areas, either local populations or recognized subspecies? 3) Again, if there is isozyme variation, how is it maintained or influenced, and what are the implications for conservation and management of these game animals?

Chapter 2

MATERIALS AND METHODS

2.1 Sampling sites, collection and storage

Red deer tissue samples were collected during three shooting seasons in Scotland, from 1979 to 1981. During 1979, six localities were sampled, from Perthshire to Caithness. Four sites were private estates, one on adjoining Forestry Commission woodland, and the final one on the island of Rhum which is managed by the Nature Conservancy Council (Figure 2 shows all Scottish sites and the years they were sampled).

In 1980, the collection area was expanded to include two Forestry Commission sites in Galloway with mixed open hill and woodland habitats, and three private estates in eastern Scotland. Red deer from four enclosed populations in the Netherlands were also tested with the cooperation of biologists there.

In 1981, red deer tissues were taken from six sites previously sampled in Scotland, representing different habitats and latitudes. These were compared with North American wapiti samples collected the same year. Wapiti were from eleven sites throughout the Pacific Northwest of the United States (Figure 3 maps the wapiti sampling area).

Samples from 139 red deer were collected in 1979; the sample size was increased to 484 deer in 1980; 64 red deer were tested with 254 wapiti in 1981. Thus a total of 943 animals from 28 localities were analysed for between 11 and 34 enzyme systems. When the sample size

Figure 2. The locations where Scottish red deer samples were collected. Symbols refer to the year, and numbers refer to the name of the site as listed in Table 14 (page 62).

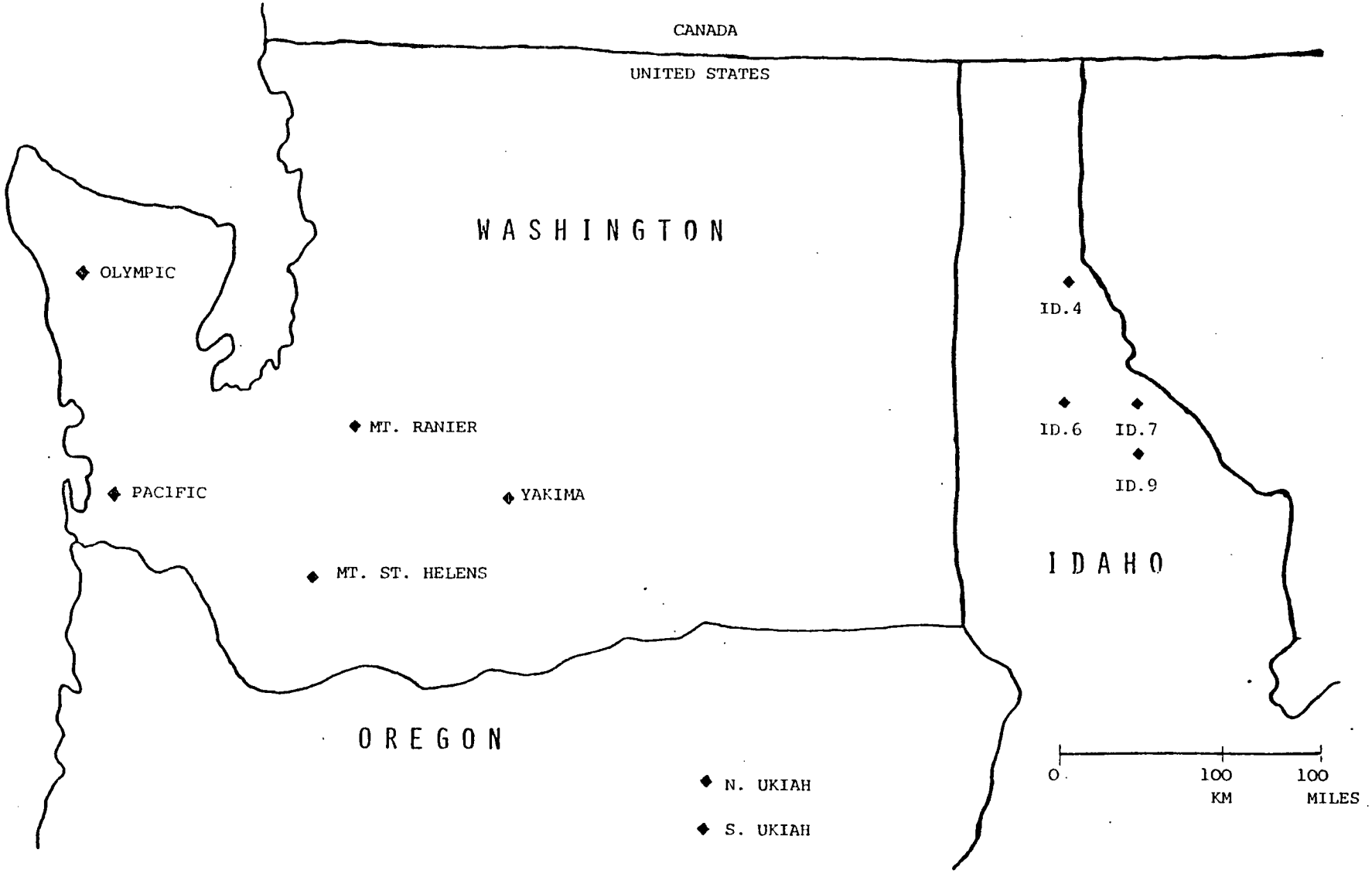


Figure 3. Collection sites in the Northwest United States for wapiti samples in 1981.

was increased in 1980, the number of enzymes tested was decreased, and three polymorphic enzymes were analysed primarily. The number of enzymes tested was again increased, to 28 loci, for the wapiti samples.

Professional stalkers and government rangers took liver, kidney, heart and striated muscle samples from each animal in the first year of the study. After initial results showed that enzyme variability could be ascertained from muscle and kidney samples, only these were collected in the subsequent two years. Muscle samples were collected from wapiti, plus liver or kidney if these were brought with the carcass by individual private hunters.

Samples were usually bagged and frozen the day the animal was shot. Bags were premarked with location numbers. Details of age, sex, weight, number of antler points in males and lactation status of females were written on the bags when this information was available. The samples were generally frozen in conventional domestic freezers at the sites until collected; they were kept frozen in an ice chest during transport, and were stored at $-20^{\circ}C$ or less until analysed by electrophoresis. All deer were analysed within eight months of when they were shot.

2.2 Electrophoretic procedure

In preparation for electrophoresis, approximately 0.5 g of frozen tissue was homogenized with an equal volume of distilled water. Homogenates were then centrifuged at 2000 rpm for 15 minutes, cooled to $10^{\circ}C$. After use homogenates were frozen at $-80^{\circ}C$ and could be retested several times if necessary. Sample test tubes were defrosted either by soaking in a $36^{\circ}C$ bath or centrifuging for five minutes.

Homogenates were discarded after five days, and new ones made from the frozen samples if needed.

Electrophoresis was performed on horizontal starch gels, essentially employing the method described by Selander et al. (1971) as modified for deer by Manlove et al. (1975). Gels contained 12% w/v hydrolyzed potato starch in a buffer solution appropriate for the enzymes tested. (Appendix 2 lists the buffers, voltages and stains for each enzyme system). The solution was heated to boiling, degassed and poured into 6 mm thick gel moulds.

When gels had formed and cooled for at least 30 minutes, they were sliced at the designated cathode end. Between 18 and 24 samples, from at least two sites, were applied along this plane of the gel. Samples were applied by dipping 1 mm thick chromatography paper into the homogenate, blotting the excess moisture and inserting the paper along the cut surface (shown in Figure 4).

Electric current was applied by extending sponges from buffer trays at each end of the gel; the buffer trays were charged from a transformer. Figure 5 shows a diagram of the apparatus. The paper inserts were removed after 15 minutes at 120 volts. The voltage was then adjusted depending on the buffer type and the intended duration of the electrophoretic run. Gels were run for either six hours during the day, or for fourteen hours overnight at much lower voltages.

To prevent enzyme denaturation due to overheating, gels were sandwiched between copper plates (after inserts were removed) which had a circulating water and anti-freeze solution at 5°C. Non-enzymatic dye markers of bromophenol blue or phenol red were used to track the progress of the run. Enzyme migration and allele separation

Figure 4. Inserting filter paper soaked in sample homogenate into starch gel. Gel is kept covered to prevent desiccation.

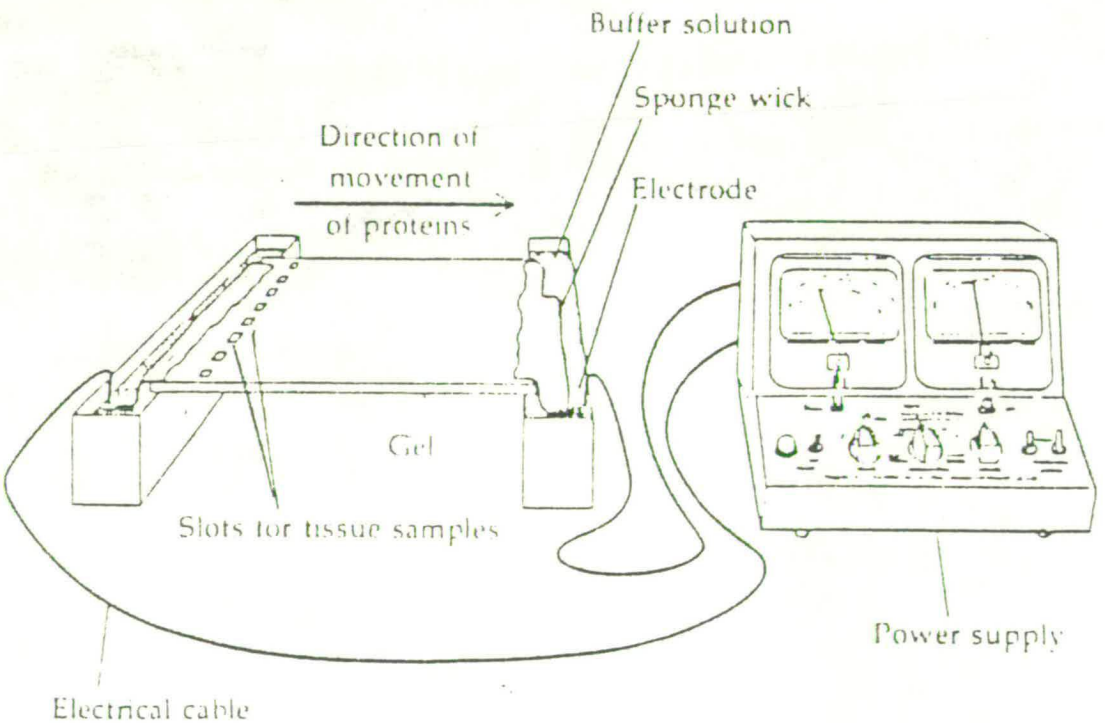


Figure 5. Horizontal electgophoretic apparatus similar to that used in the study. Reprinted from Hartl (1980) with permission.

was similar in both day and night electrophoretic runs.

When dye markers had migrated to the cathodal sponge, the run was terminated. After excess moisture was blotted from the gels, they were cut longitudinally into slices of 1 m to 1.5 m thickness, depending on the number of enzymes to be analysed for a particular buffer system.

Enzyme stains were prepared just before the electrophoretic run was completed, and photoactive chemicals were added after gel slicing. Two staining methods were used: slices were either soaked in a stain bath or covered with a 2% agar stain solution which cohered to the gel surface as it cooled. The latter method was favoured toward the end of the study as it conserved staining chemicals. Most stains were incubated at 38°C in the dark, though some enzymes (SOD, 6PGD, PEP) required special staining conditions (Harris and Hopkinson, 1976).

When the enzyme reaction bands had resolved, usually within five to thirty minutes of staining, slices were destained in a 10% acetic acid solution. The results were then scored and if electrophoretic variants were observed, the gels were either photographed or stored in a cold room for comparative purposes.

2.3 Analysis of data

Data analysis began with the description of the electrophoretic banding pattern for each deer under each enzyme stain. In the case of multiple bands, it was necessary to determine whether they were the products of more than one locus coding for the same enzyme or allelic variants at a single locus. This was inferred from the quaternary structure of the enzyme (Darnall and Klotz, 1975) and from banding

patterns pictured for other mammals (Harris and Hopkinson, 1976). The nomenclature used to describe isozymes followed the system outlined by Allendorf and Utter (1979). Necessarily elaborate, if results for particular loci are to be extrapolated to other studies, the procedure for naming isozymes is detailed in the first section of the next chapter.

The gene frequencies of polymorphic loci were the primary means of comparison between red deer and wapiti populations. A locus was considered polymorphic if the frequency of the most common allele did not exceed 99% (Hartl, 1980; Smith et al., in press). At a higher frequency than this, the common allele was considered to be at fixation, and the particular enzyme locus monomorphic.

Statistical analysis began simply by counting the genotypes of polymorphic loci at each locality and calculating the gene frequency. The observed genotype frequencies were then compared with Hardy-Weinberg expectations by a chi-square test. Genetic differentiation among red deer and wapiti populations was determined by testing the null hypothesis that gene frequencies between localities were not significantly different. Allele frequencies of polymorphic loci were compared by two methods: chi-square contingency tables and log-liklihood ratio tests (Sokal and Rohlf, 1969).

The amount of differentiation among red deer and wapiti was determined by calculating the fixation index (F_{st}, Wright, 1943) and the coefficient of gene differentiation (G_{st}, Nei, 1975); these formulae in fact give the same results when there are only two alleles at any polymorphic locus, as was the case with wapiti and red deer. The absolute genetic differentiation (Dm, Nei, 1975) was initially calculated for Scottish red deer populations. When these populations

were compared with Continental red deer or North American wapiti, a hierarchical gene diversity analysis was used (NEGST, Chakraborty et al., 1982) to determine the proportion of variation attributable to different levels of social organization - individuals, populations, subspecies, etc.

Three indices are commonly used to compare the amount of genetic variation in disparate organisms. The mean heterozygosity (H) is the proportion of the population that is heterozygous over all loci tested, both polymorphic and monomorphic. P is the percentage of polymorphic loci in a population, and A is the average number of alleles per locus. A Mann-Whitney U-test was used in comparing these indices between populations. To test for evidence of selection on particular isozymes, a two way analysis of variance (Harvey, 1960) compared enzyme variation with quantitative characters such as weight and age, antler points and lactation status.

Last and least significant, topologies of relationship between populations were constructed from the gene frequency data. As with most of the statistical analyses, two alternative methods were used. Dendrograms employed the measure of genetic distance suggested by Nei (1972), as this method considers both monomorphic and polymorphic loci, and is thus comparable with previous deer studies. For the same data, phylogenetic trees were constructed using the program described by Felsenstein (1981), as this second technique includes confidence intervals for branch lengths.

Chapter 3

DESCRIPTION OF POLYMORPHIC ENZYMES

3.1 Interpretation and notation

The genetic interpretation of electrophoretic patterns of isozymes followed the principles outlined by Allendorf et al. (1977). It was based on several factors: tissue distribution of the pattern and inferred enzyme structure (Darnall and Klotz, 1975); the frequencies of different allelic variants and their agreement with Hardy-Weinberg expectations; and comparison of isozyme banding patterns with other deer species (Ryman et al., 1980; Smith et al., in press) and other mammals generally (Harris and Hopkinson, 1976).

The nomenclature used to designate enzyme loci and alleles employs the system proposed by Allendorf and Utter (1979). The common abbreviation is used for each enzyme (i.e. Isocitrate dehydrogenase, IDH). When more than one locus is analysed for a particular enzyme, a hyphenated numeral is added and loci are numbered starting with the least anodal form (i.e. IDH-2 denotes the more anodal, or further migrating, locus of IDH). In addition to being internally consistent, this procedure conforms with many current electrophoretic surveys of natural populations.

In a rapidly developing discipline, however, some nomenclature inconsistencies in the literature are inevitable. For example, the supernatent form of IDH, designated as IDH-2 in red deer and moose, is called IDH-1 in mice, for historical reasons. Similarly, malic enzyme is abbreviated ME in most deer studies, though MOD is the notation

used in mice. When such discrepancies occur, an abbreviation is not used for the animal which is being compared with red deer or wapiti.

Allelic variants within particular loci are also designated to their electrophoretic mobility (Allendorf and Utter, 1979). One banding pattern - generally that of the more common allele if the enzyme locus has not been previously described - is designated as 100 and the other alleles are given numerical values representing the electrophoretic mobilities of their banding patterns relative to this unit distance. For example, the band designated as IDH-2 (125) migrates 25% further than IDH-2 (100) for a specified gel buffer.

Of 34 enzyme loci analysed, eight proved polymorphic in either Scottish red deer, North American wapiti, or both. These were GPI-1, IDH-2, ME, MPI, PEP-2, PGM-1, PGM-2, and SOD-1. For each polymorphic locus, there follows a) the enzyme abbreviation, classification number (E.C.) and a brief outline of its metabolic function, b) a description of the banding patterns observed, c) the genetic interpretation of the banding variation in light of the quaternary structure of the molecule, d) evidence of polymorphism at the locus in other deer species, and e) confirmation of its genetic inheritance through progeny testing and/or pedigree studies in other mammals - mostly mouse and man.

3.2 Isocitrate dehydrogenase, IDH (E.C. 1.1.1.42)

IDH was polymorphic in red deer from all Scottish localities sampled. The enzyme is part of the citric acid cycle, and its function is to catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (Chen, et al., 1972). There are at least three different isocitrate dehydrogenase enzymes in most plants and animals,

one of which is dependent on the cofactor NAD for its reaction, the other two on NADP (Turner, et al., 1974). The latter form of IDH was tested in red deer. (NAD is the conventional abbreviation for nicotinamide adenine dinucleotide; NADP stands for nicotinomide adenine dinucleotide phosphate.)

NADP-dependent isocitrate dehydrogenase has two genetic loci (Bell and Baron, 1968), one coding for the mitochondrial enzyme (IDH-1) and the other for the cytoplasmic form (IDH-2). Though both were analysed, only IDH-2 (also called the soluble or supernatent locus by some authors) showed variation in this study. Henderson (1968) analysed supernatent IDH in several mammalian species; he found two alleles in mice and confirmed the variation as genetic by progeny testing. Two more supernatent IDH alleles have been found subsequently, in Japanese wild mice (Minezawa et al., 1976, 1978).

Three different banding patterns were observed for supernatent IDH in red deer (Figure 6). Two were single bands of different mobilities, presumed homozygotes IDH-2 (100/100) and IDH-2 (125/125) respectively; the other was three-banded, the heterozygote IDH-1 (100/125). This banding pattern is characteristic of dimeric proteins - those with two polypeptide chains in their quaternary structure - and the intermediate band, or heterodimer, characterizes enzymes of this molecular structure. Recently, a similar fast allele has been found in supernatent IDH in white-tailed deer (Smith, et al., in press).

3.3 Superoxide dismutase, SOD (E.C. 1.15.11)

Superoxide dismutase was also polymorphic in all Scottish red deer populations sampled initially, though when the survey was

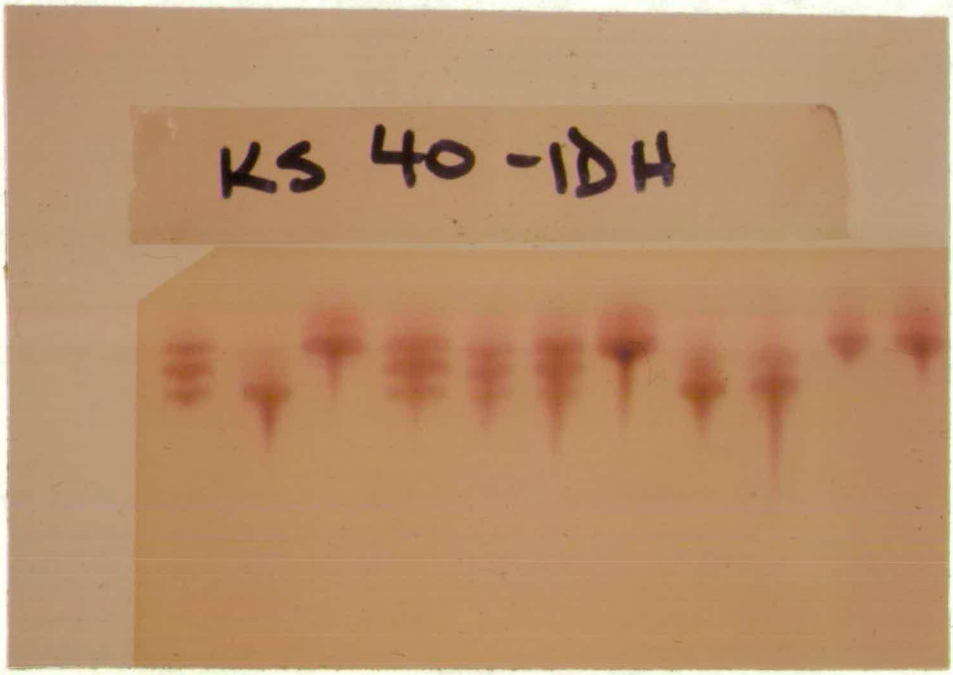


Figure 6. Variation in Isocitrate dehydorgenase (IDH-2). At this dimeric locus, homozygotes are single banded, and heterozygotes (IDH-2 100/160) have three bands.

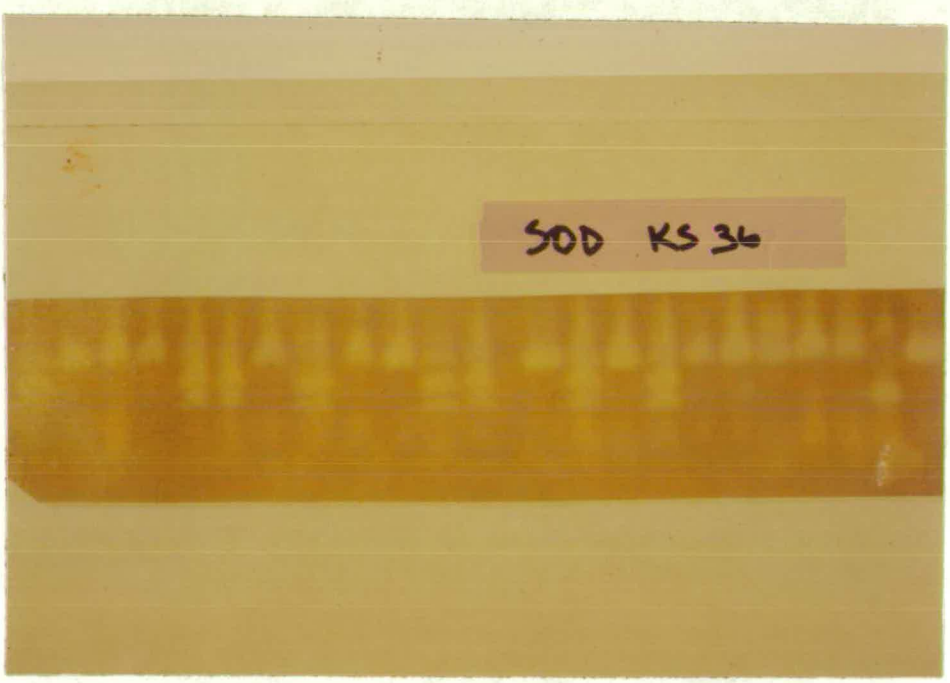


Figure 7. Superoxide dismutase (SOD-1) proteins migrate to the cathode in acid citrate buffer. Dimeric, with single banded homozygotes: SOD-1 (-100) predominates in red deer, and SOD-1 (-225) predominates in wapiti. Three banded hets.

extended to Galloway, the two populations there were at fixation. SOD is also dimeric, though it migrates cathodally on the same gel buffer as used for IDH, and stains differently.

Light or achromatic areas in heavily stained starch gels were first described by Brewer (1967), and he postulated that this was the result of the reduction of tetrazolium catalyzed by light. (The enzyme has been called tetrazolium oxidase for this reason, as well as indophenol oxidase.) In a series of experiments on anaerobic and aerobic organisms, McCord et al. (1971) concluded that the function of SOD was the removal of oxygen-free radicals, which are produced by air-breathing organisms and are detrimental to living cells.

Beckman et al., (1973), examining a variety of human tissues and fluids, identified two SOD enzymes, mitochondrial and cytoplasmic. Both were tested in tissue samples from red deer, and the cytoplasmic locus (SOD-1) was polymorphic, again yielding three banding patterns. (Figure 7). Brewer (1967) found similar variation within a human family, first suggesting the SOD polymorphism was inherited. The same banding pattern, indicating two allele segregation at a dimeric locus, was published by Baur et al., (1969), in a random survey of 197 dogs at a veterinary clinic. The authors noted significant differences in gene frequency between breeds.

Welch and Mears (1972) found a rare variant of the cytoplasmic SOD locus among inhabitants of the island of Westray in the Orkneys of Scotland. The three-banded heterozygote occurred in 12 of 406 islanders, a gene frequency of 1.48% (if both parents were sampled, children were not). Noting that the rare allele might have increased in frequency by inbreeding on an island with a population of about 750, they predicted that the variant might also be found, though at a

lower frequency, in Scandinavia, the origins of Westray settlers. Apparently independently, Beckman et al., (1973) found the rare SOD variant in Scandinavia. Sampling 3512 people, the rare allele occurred at a frequency of 0.4% in northern Sweden, 1.1% in northern Finland and at 2.46% in one valley bordering the two countries.

3.4 Glucose phosphate isomerase, GPI (E.C. 5.3.1.9)

A rare variant of GPI was found only in Scottish red deer populations, and there at low frequency. The enzyme was first described by Lohmann (1933) and its function, the reversible conversion of glucose-6-phosphate to fructose-6-phosphate, was confirmed by Tsuboi et al. (1958).

Carter and Parr (1967), using hàemolysates and muscle extracts from mice, found genetic variation at a single dimeric autosomal locus, and DeLorenzo and Ruddle (1969) confirmed the presence of two GPI-1 alleles. In wild mice from Somerset, Padua et al. (1978) found two more GPI-1 alleles, one migrating faster and the other slower than the two variants previously described. A second GPI locus has also been described in mice (Peterson and Wang, 1978). Its postulated function is regulatory; the two variants appear to control the expression of the structural, GPI-1 locus.

In red deer, the three banding patterns observed for GPI-1 suggest similar variation of a dimeric protein (Figure 8). However, the banding pattern of GPI is more complex than that of IDH-2 or SOD-1, with three-banded homozygotes and the heterozygote having five or more bands. Ryman et al. (1977) found the same pattern in Scandinavian moose. After further testing (Ryman et al., 1980), they suggested that the multiple bands were the product of two overlapping

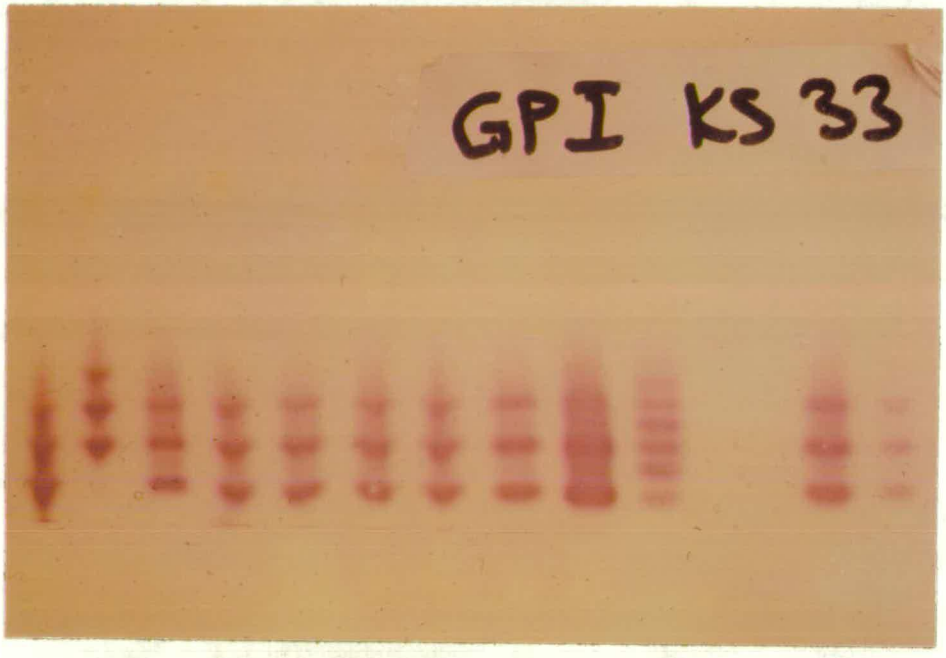


Figure 8. Polymorphic pattern for Glucose phosphate isomerase. Two loci, GPI-1 polymorphic; GPI-2 monomorphic. Three banded homozygotes (fast allele, second from left) and six banded heterozygotes.

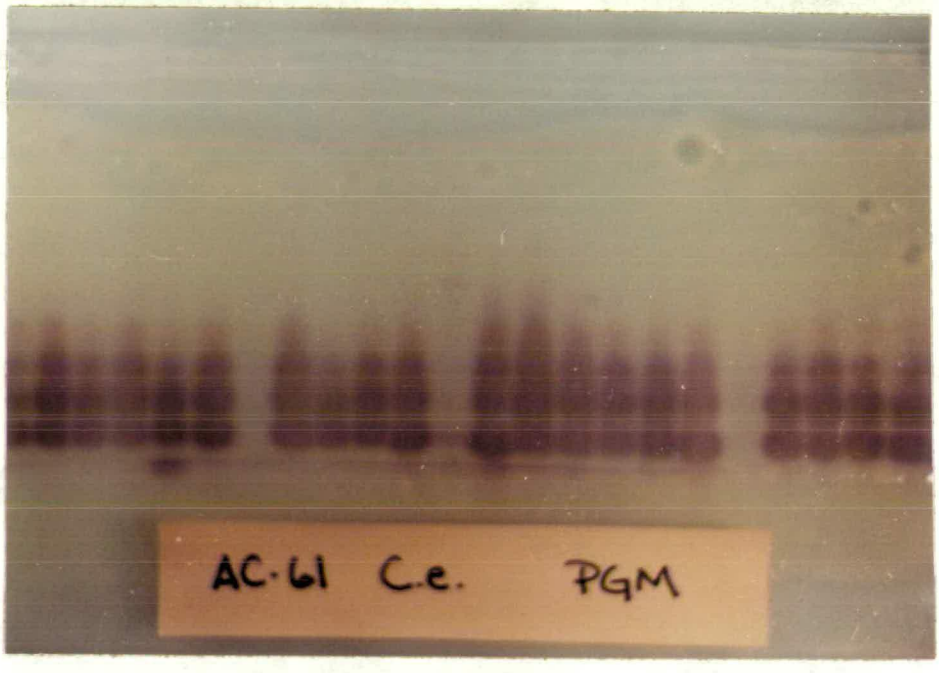


Figure 9. Phosphoglucomutase has two loci. PGM-1 is monomeric, with a single slow variant shown. PGM-2, the upper bands, shows no variation on this gel.

autosomal loci and their secondary isozymes - the rare allele of the GPI-1 locus having the same migration rate as the allele of monomorphic GPI-2 locus.

The three banding patterns found in Scottish red deer conform with this interpretation, as the rare homozygote (GPI-1 160/160) has been found in a few individuals and the frequencies from all localities concur with Hardy-Weinberg expectations (Chapter 4). Whether the triple-banded homozygotes of GPI are due to two overlapping loci for the buffer used to analyse the enzyme, or are solely secondary enzyme products, it does appear that the variation is genetic, with two segregating alleles, GPI-1 (100) and GPI-1 (160).

Rare allelic variants of GPI (also called PHI, phosphohexose isomerase) have been found in several surveys of human populations around the world (Detter et al., 1968; Blake et al., 1971; Omoto and Blake, 1972). Their occurrence in particular ethnic groups (Fitch et al., 1968) and especially pedigree studies on two Japanese families (Nakashima, et al., 1973) leave little doubt that GPI variation is inherited, and the latter work suggests that rare alleles may be associated with GPI deficiency and problems of glucose metabolism.

3.5 Phosphoglucomutase, PGM (E.C. 2.7.5.1)

Phosphoglucomutase is widely distributed in mammalian tissues and plays an important role in carbohydrate metabolism, catalyzing the transfer of a phosphate group between the 1- and 6- positions of glucose (Spencer, et al., 1964). The genetic interpretation of PGM variation has been complicated by two factors: different degrees of enzymatic activity at each locus in different tissues (McAlpine, et al., 1970), and the occurrence of secondary isozymes depending on the

age and preservation of the samples (Fisher and Harris, 1972; Green, 1981).

Three PGM loci have been documented in humans, and each show allelic variation (Harris and Hopkinson, 1976). In mice, two PGM loci have been reported: PGM-1 has at least four alleles, while two alleles have been found at PGM-2 (Selander et al., 1969; Shows et al., 1969; Chapman et al., 1971).

In red deer, two PGM loci were scorable, and both showed variation in Scotland. As PGM has a monomeric quaternary structure (the amino acids which constitute the enzyme are in a single polypeptide chain), there was no intermediate band in heterozygotes (Figure 9). The banding patterns at each locus suggest the segregation of two alleles, PGM-1 (100, 90) and PGM-2 (100, 70) respectively.

Selander et al. (1969) found the same pattern of variation in the European house mouse (Mus musculus), with variants occurring at both loci in one subspecies (M.m. musculus) and not in another (M.m. domesticus). In deer species, PGM variation has been observed in moose (Ryman et al., 1980) and in white-tailed deer (Manlove et al., 1976; Ramsey et al., 1979). In white-tails, variation which was detected but could not be scored at a second, more anodal locus (possibly homologous to red deer PGM-2) has recently been confirmed (Smith et al., in press).

3.6 Malic enzyme, ME (E.C. 1.1.1.40)

Malic enzyme, variable in several Scottish localities, catalyzes the carboxylation of malate to pyruvate (Ochoa et al., 1947) as well as oxalate to pyruvate (Ochoa et al., 1948), again in the presence of

NADP. Both mitochondrial and cytoplasmic forms have been identified (Frankel, 1971), though only the cytoplasmic enzyme was analysed in red deer, as the mitochondrial form was not present in muscle and faintly visible in kidney.

Three ME banding patterns were found in red deer: two homozygotes, (ME 100/100) and (ME 125/125), and the presumed heterozygote (ME 100/125) with a wide, blurred intermediate band. Shows and Ruddle (1968), working with fresh mouse samples, found five intermediate bands in heterozygotes indicating a tetrameric quaternary structure for cytoplasmic malic enzyme. The same group (Shows et al., 1970) also confirmed the inheritance of these variants with progeny testing on wild type and inbred strains. Variation at both ME loci has been found in macaques (Cohen and Omenn, 1972), with a blurred intermediate band for heterozygotes similar to that found in red deer.

As with IDH, there is an NAD dependent malic enzyme, though it goes by another name, malate dehydrogenase (MDH). Gyllensten et al., (1982) have found an MDH-1 variant in one of sixteen red deer populations sampled in continental Europe; no polymorphism at either MDH locus was observed at Scottish localities.

Several factors make interpreting ME banding patterns difficult. The presence of ME-2 often depends on how well tissues are homogenized and the cell walls broken down. The tetrameric structure of the enzyme often causes the tightly packed multiple bands of heterozygotes to appear as a blur. Finally, because trace impurities of NAD are often present in commercially available NADP, malate dehydrogenase sometimes stains on gel slices intended to detect ME, obscuring some of the malic enzyme bands.

3.7 Mannose phosphate isomerase, MPI (E.C. 5.3.1.8)

Polymorphism for the enzyme mannose phosphate isomerase was found in all wapiti populations, though not in red deer. MPI catalyzes the reversible conversion of mannose-6-phosphate to fructose-6-phosphate (Slein, 1955), requiring the same substrate and giving the same reaction products as GPI. Gottschalk (1947) first suggested that two unique enzymes produced the common intermediate fructose-6-phosphate. Gray and Noltmann (1968) eventually isolated and measured MPI.

Unlike GPI, mannose phosphate isomerase is a monomer (McMorris et al., 1973). Thus, presumed heterozygotes in wapiti (MPI 100/160) are double-banded and homozygotes have single bands (Figure 10). There is the possibility of a third, faster migrating band; however, its rarity and overlap with a secondary isozyme of the common allele (MPI 100) will require further work - perhaps with a large number of fresh samples which have fewer secondary isozymes - to confirm whether it is an additional allele. Only the two readily identifiable alleles were included in the gene frequency analysis.

Moose and mice both show a similar slow variant for MPI (Ryman et al., 1980; Nichols et al., 1973). In white-tailed deer three alleles have been described (Smith, et al., in press); the fastest allele was most common and the slower alleles had mobilities similar to those of moose and wapiti.

3.8 Peptidase, PEP-2 (E.C. 3.4.11)

Lewis and Harris (1967) have described five peptidase enzymes which rely on different peptides for their reaction, and Rapley et al., (1971) have added two others. One of these peptidase enzymes was analysed in red deer and wapiti, using the tripeptide leucyl-

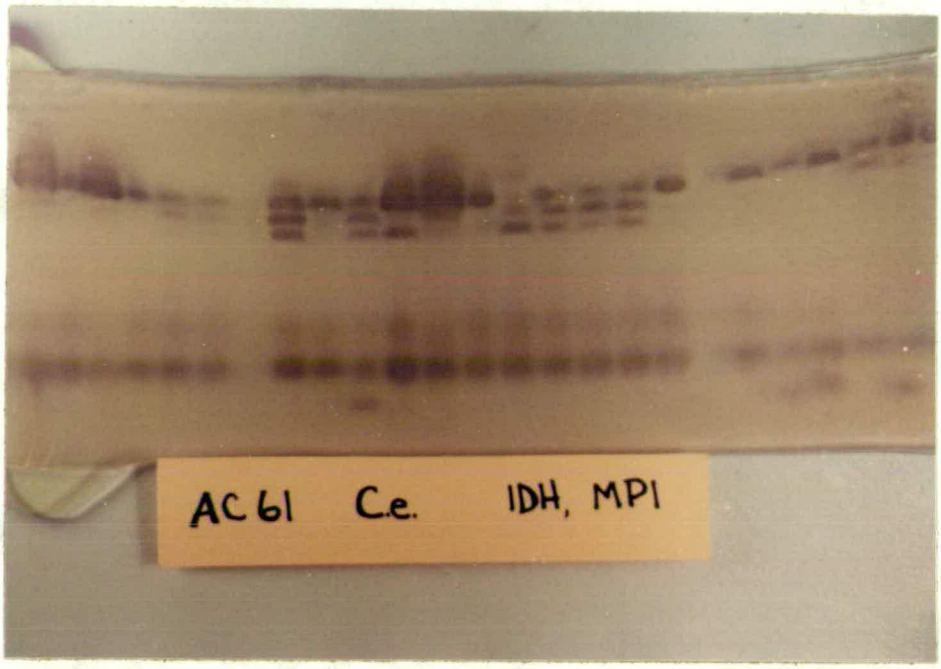


Figure 10. Mannose phosphate isomerase is a monomeric locus with single banded homozygotes and two banded heterozygotes. Three heterozygotes are shown, one in the center and two at the right. IDH-2 variation is shown above on the same gel.

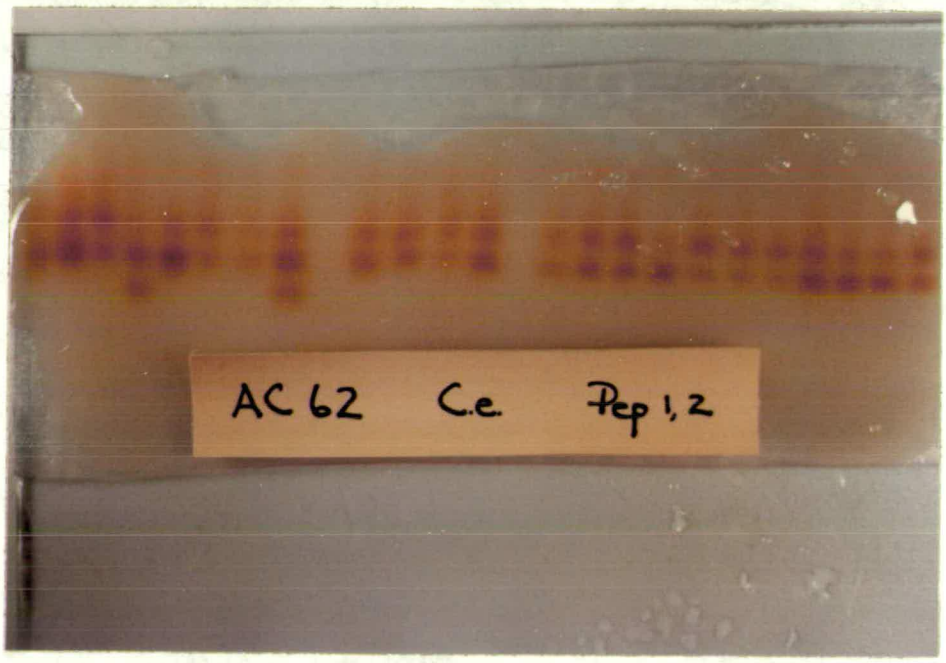


Figure 11. Peptidase (leu-gly-glycine). Monomeric, with two banded homozygotes with three banded heterozygotes (PEP-2 100/82) two of which are shown on the gel.

glycyl-glycine as the substrate. This peptidase is equivilent to PEP-2 described in mice (the notation adopted here for deer), and PEP-B in man.

PEP-2 polymorphism was found only in wapiti populations and not in red deer. Two alleles were detected in wapiti (PEP-2 100, 80). As the enzyme is a monomer (Rapley et al., 1971), there was no intermediate band observed in heterozygotes. PEP-B 2-1, pictured by Lewis and Harris (1967), closely resembles the banding pattern found in wapiti heterozygotes (Figure 11). Polymorphism for the same peptidase has also been found in moose (Ryman et al., 1980) and white-tailed deer (Smith et al., in press), both species also having two alleles.

Six peptidase-B alleles have been detected in humans (Harris et al., 1974), most of them rare. Testing the offspring of a mother with the common allele and a father homozygous for one of the rare alleles, Rapley et al. (1971) found all six children were heterozygous, demonstrating the inheritance of the enzyme.

Chapter 4

ELECTROPHORETIC VARIATION IN RED DEER

4.1 Scottish populations, 1979

Of the six enzymatic loci which were polymorphic in Scotland, three (IDH-2, ME and SOD-1) showed variation at all localities sampled in 1979. GPI-1 was polymorphic at every site sampled that year except Caithness, and as the overall gene frequency of the less common allele, GPI-1 (160), was 7.1% ($p = 0.929$), its absence was likely the result of small sample size ($n = 20$) that year. When the sample size was slightly increased in 1980 ($n = 29$), the fast allele was found in Caithness, though not at Loch Laggan or Strathmashie. At both PGM loci, the alternate alleles were quite rare, only exceeding 5% at one locality and absent in several.

The genotypes of all polymorphic loci were first tested for agreement with Hardy-Weinberg expectations at each sampling locality. This is demonstrated below for two enzymes in the Rhum population, with differing results.

RESULTS FROM DATA SET: GPI RHUM

GPI-1	(100/100)	(100/125)	(125/125)	Banding pattern mobilities
	35	5	1	Observed genotype numbers
	0.854	0.122	0.024	Genotype frequencies
	0.915	0.085		Gene frequencies
	34.299	6.402	0.299	Expected genotype numbers
	0.014	0.307	1.646	Deviations from expected

THE HARDY-WEINBERG CONST = 1.9673

WITH 1 DEGREE OF FREEDOM (COR.COEFF.=3.84)

IN HARDY-WEINBERG EQUILIBRIUM.

RESULTS FROM DATA SET: IDH RHUM

IDH-2	(100/100)	(100/125)	(125/125)	
	12	13	16	N = 41
	0.293	0.317	0.391	P + H + Q = 1
	0.451	0.549		p + q = 1
	8.348	20.305	12.348	p^2N, 2pqN, q^2N
	1.598	2.628	1.080	$(Obs.- Exp.)^2$ /Expected

THE HARDY-WEINBERG CONST = 5.3065

WITH 1 DEGREE OF FREEDOM (COR.COEFF.=3.84) d.f. = 3 - 1 - 1 = 1

NOT IN EQUILIBRIUM.

Table 8. Genotype and gene frequencies for two loci of Rhum deer sampled 1979.

The IDH-2 locus on Rhum was the only case of a polymorphic locus significantly different from Hardy-Weinberg proportions in local populations (Table 9). By contrast, when the six localities were pooled and the Scottish population tested as a single unit, two of the six polymorphic loci (ME and SOD) were not in Hardy-Weinberg equilibrium, and the difference at two others (GPI-1 and IDH-2) approached significance - in all cases due to a deficiency of heterozygotes. The results suggested that the Scottish red deer population was genetically subdivided, though it remained to be more rigorously tested.

To determine whether the decrease in overall heterozygosity was a result of local gene frequency differences, contingency tables for polymorphic loci were constructed from the gene frequencies and sample sizes at each locality (Appendix 4A). These test the hypothesis that the differences in gene frequency at the six sites do not significantly differ from proportions of six random samples drawn from the overall population. When, in the case of uncommon alleles, there were several expected values less than five, the populations with

similar frequencies were pooled and the degrees of freedom reduced accordingly (Sokal and Rohlf, 1969). The results, again in Table 9, show significant heterogeneity at four of the six polymorphic loci.

LOCATION	IDH-2	GPI-1	SOD-1	PGM-1	PGM-2	ME
Caithness	0.475	1.0	0.857	0.875	0.950	0.971
(n = 20)	1.839	0.0	1.037	0.408	0.055	0.016
Ross-shire	0.177	0.968	0.903	0.984	0.984	0.813
(n = 31)	1.442	0.344	0.034	0.008	0.008	1.031
Loch Laggan	0.132	0.882	0.658	1.0	1.0	0.816
(n = 21)	1.815	0.302	0.062	0.0	0.0	0.016
Strathmashie	0.423	0.962	0.929	1.0	1.0	0.917
(n = 14)	0.585	0.021	0.083	0.0	0.0	0.099
Rhum	0.451	0.915	0.890	1.0	0.988	0.988
(n = 41)	5.307*	1.967	0.654	0.0	0.006	0.006
Perthshire	0.286	0.893	0.857	1.0	1.0	0.760
(n = 28)	0.070	1.797	2.361Y	0.0	0.0	0.377
Pooled populations (d.f.) p <	3.391 (1)ns	3.061 (1)ns	5.138 (1)*	0.061 (1)ns	0.026 (1)ns	5.390 (1)*
Heterogeneity (d.f.) p <	24.20 (5)***	5.51 (3)ns	13.04 (5)*	27.02 (1)***	5.06 (1)ns	23.06 (5)***
H_t	0.438	0.119	0.256	0.046	0.026	0.215
F_{st}	8.3%	3.1%	6.1%	9.1%	2.4%	6.8%

Table 9. Gene frequencies for polymorphic loci and beneath each, deviations from Hardy-Weinberg expectations. Asterisks denote level of significance, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Y after a chi-square value denotes that Yates' correction for continuity was used. Below the line are the heterogeneity and fixation index of each locus.

In calculating the cumulative heterogeneity, chi-square values from independent sources may be added together and the degrees of freedom corresponding to that sum are equal to the sum of the degrees of freedom of the independent comparisons (Sokal and Rohlf, 1969). Using this procedure (which assumes the enzyme loci chosen randomly are independent), the heterogeneity over all polymorphic loci was

highly significant (X^2=97.89 d.f.=25 p<0.001). The same Scottish populations were also compared using a log likelihood ratio (G) test (Sokal and Rholf, 1969), with the same highly significant result (shown in Table 11, next section).

To get a better understanding of the genetic structure of the subdivided Scottish deer population, the fixation index (F_{st}) was calculated at each polymorphic locus. The fixation index technically measures the amount of differentiation in the subpopulations relative to a hypothetical group of homozygous subpopulations (i.e. fixed at p or q), but with the same average gene frequency as measured from the actual subpopulations.

For the 1979 Scottish data, PGM-1 and IDH-2 showed the greatest divergence between localities. In the former case this was due to the higher gene frequency of the alternate allele (PGM-1 90) at Caithness, and in the latter it was due to the wide range of frequencies between 0.177 and 0.475 across localities. The range in gene frequencies at the IDH-2 locus is greater still in red deer over a wider geographic area, as will be shown in the next section. The predominant allele throughout Scotland (IDH-2 125) is less common in most deer populations sampled in continental Europe. For consistency in later comparison, the gene frequencies of IDH-2 in Table 9 are for the alternate allele ($p + q = 1$), and therefore are less than 0.5. Finally in Table 9, it is worth noting that the significance of the contingency chi-square results for each locus reflect the magnitude of the fixation indices immediately below them. Workman and Niswander (1970) have pointed out that the two statistics are closely related.

The summary statistics (Table 10) enable comparison of the Scottish red deer population with the gene frequency data from

populations of other organisms. Because no more than two alleles were observed at any locus in red deer or wapiti populations, the relationship between the percentage of polymorphic loci (P) and the number of alleles per locus (A) is constant: A = 1 + P. Therefore, only P is listed in later comparisons. The two measures of heterozygosity are also related: H is measured over all loci, regardless of variation, H_s is the subpopulation heterozygosity based only on the polymorphic loci. H_s is retained here for the calculation of the fixation index (F_{st}).

LOCATION	P	A	H%	Hs
Caithness	0.147	1.147	3.3	0.1856
Ross-shire	0.176	1.176	2.6	0.1492
Loch Laggan	0.118	1.118	3.5	0.1979
Strathmashie	0.118	1.118	2.5	0.1409
Rhum	0.147	1.147	2.6	0.1490
Perthshire	0.118	1.118	3.6	0.2018
	0.137	1.137	3.0	0.1707 H_s 0.1833 H_s 6.87% F_{st}

Table 10. Summary statistics for 1979 Scottish red deer analysis. H_t is the average of the total heterozygosity for polymorphic loci in the previous table.

In a review of electrophoretic studies where fourteen or more loci were analysed, Nevo (1978) found that, among 46 mammal species, H and P values averaged 3.6% and 0.147 respectively. The Scottish red deer tested in 1979 (H = 3.0% and P = 0.137) thus fall close to the average for other mammals. Baccus et al. (1983) have recently extended the comparison to 53 mammals, adding several large grazing species not previously considered (including bison and several deer species), and yielding averages (H = 3.3% and P = 0.128) which even

more closely approximate the enzymatic variation found initially for red deer in Scotland.

It must be added, however, that these results may be underestimates of the average genetic variation in Scottish red deer as blood proteins were not tested. In humans, blood proteins are more polymorphic on the average than enzymes (Harris, 1966), though this may not be the case in animals generally (Nei, 1975). Anecdotal evidence, from blood in muscle and liver tissues, suggest that there are at least two haemoglobin banding patterns in these red deer, and brief experiments when blood samples were available confirmed that the three transferrin alleles found in Scandinavian red deer populations (Gyllensten et al., 1980) also occurred in sera of Scottish deer.

The relative genetic divergence (F_{st} = 0.068) between the six Scottish deer populations was moderate by Wright's standards (Hartl, 1980), whereas the absolute genetic divergence (Dm = .0027) was quite low. In other words, 6.8% of the genetic variation detected was due to differences between localities, and the remainder was due to differences between individuals within localities. This is similar to the relative divergence between the three major human races (Negroid, Caucasoid, and Mongoloid) as measured over 35 loci (Nei, 1975). When both polymorphic and monomorphic loci are considered, these red deer varied over only 0.27% of their genome as surveyed electrophoretically. Although this difference seems very small, it is comparable to that found by Ryman et al. (1980) in Scandinavian moose populations (Dm = 0.0021), and it is greater than that found in red deer from elsewhere, as shown in the next section.

4.2 Comparison with other northern European red deer populations

In collaboration with population geneticists in Sweden, the 1979 Scottish samples were compared with 441 red deer from continental Europe. The Continental samples came from sixteen localities in three countries (detailed in Appendix 4B), though they were predominantly from Sweden.

Deer from different localities were analysed on the same starch gels, so the common migration rates of alleles for all loci could be confirmed. (The comparison was undertaken while I was a visiting researcher at the University of Stockholm in the summer of 1980). Representatives of four presumed subspecies were analysed, as well as Swedish red deer thought to be of hybrid stock as a result of reintroductions. The red deer were compared at all 34 enzyme loci analysed in the Scottish samples. Transferrin was also tested in the Scandinavian deer, but as blood samples were not available from Scotland or Germany, this protein locus was not included in the direct comparison between subspecies.

Neither PGM locus, polymorphic in Scotland, showed any variation in the continental deer samples. GPI was also monomorphic except for one enclosed population in Sweden, and it was subsequently learned that the Eriksberg enclosure was partly stocked with red deer from Britain (Gyllensten et al., in prep.) Among loci which were polymorphic throughout Scotland, all were monomorphic in some of the sixteen continental populations: SOD-1 was fixed at eleven localities, ME at five, and IDH-2 at three. The only locus which showed variation on the continent but not in Scotland was MDH-2, which was polymorphic at just one site, the German national park Bayerische Wald.

As was the case with the Scottish sampling sites, the gene

frequencies from the vast majority of continental localities fell within Hardy-Weinberg expectations. Overall, only 6% of 66 genotype tests on polymorphic loci significantly differed from the binomial distribution.

Gene frequency heterogeneity was evaluated hierarchically using a log likelihood (G) test (Sokal and Rohlf, 1969). Comparisons were made between localities within each subspecies, between native Swedish red deer and presumed hybrids, and between the four presumed subspecies (Table 11). The cumulative results (summed over all polymorphic loci, as done previously in the chi-square tests) showed significant heterogeneity between localities within the Cervus elaphus elaphus subspecies, as was found within C.e. scoticus. The Swedish hybrid populations showed the same highly significant result, as might be expected for animals recently established from different sources. In contrast, there was no difference between the two sites sampled from Norway (C.e. atlanticus), and only one C.e. germanicus was analysed.

	LEVEL OF COMPARISON				
Cervus elaphus	scoticus	elaphus	atlanticus	Hybrids	Subspecies
d.f.	5	6	1	5	3
GPI-1	10.35	0	0	55.65***	41.02***
IDH-2	25.45***	31.22***	0	15.69*	464.89***
ME	26.88***	8.70	0	19.34**	52.99***
MDH-2	0	0	0	0	23.95***
PGM-1	18.84**	0	0	0	12.76**
PGM-2	5.83	0	0	0	8.49*
SOD-1	12.85*	39.06***	0	61.06***	49.38***
Transferrin	-	23.39***	0	22.28***	-
d.f.	30	24		25	21
G	100.20***	102.37***	0	174.14***	53.48***

Table 11. Log likelihood ratio tests for gene frequency heterogeneity. Degrees of freedom depend on number of groups compared (d.f.= n -1), where n is the number of populations in the first four columns and number of subspecies in the final column.

When all C.e. elaphus localities were treated as a single population (sites A-G) and compared with all presumed hybrid populations (sites H-M) the difference in gene frequencies was again highly significant. Lastly, when the localities for each presumed subspecies were pooled, the heterogeneity between subspecies was also highly significant. As can be seen in the body of Table 10, in each case where cumulative heterogeneity was found, the frequencies of the majority of polymorphic loci were also significantly different. These results indicate that the genetic subdivision detected in Scottish red deer characterizes these continental deer populations as well.

As with the gene frequency heterogeneity, the relative genetic diversity could also be treated hierarchically (Nei, 1975; Chakraborty, 1982). As Nei points out, his measure of relative gene diversity (G_{st}) is the same as Wright's fixation index (F_{st}), when there are only two alleles at a polymorphic locus (Nei, 1975, p.151), as was the case with these red deer. The G_{st} results, detailed for each polymorphic locus in Table 12, cumulatively approximate the source of the gene frequency variation: 73% between individuals within sampling localities, 5% between localities within subspecies, and 22% between subspecies.

This suggests that, although deer from local populations are not homogenous, these differences are outweighed over larger geographic areas. The absolute genetic divergence between the European red deer sampled remains low (Dm = 0.0028), as expected with only one additional polymorphic locus at one locality. However, the genetic subdivison found between local red deer populations appears to be magnified between subspecies.

	RELATIVE GENE DIVERSITY (G_{st})		
	Between subspecies %	Between populations %	Within populations %
GPI-1	3.3	3.0	93.7
IDH-2	39.1	4.6	56.2
ME	2.4	5.3	92.3
PGM-1	1.2	9.0	89.8
PGM-2	0.7	2.4	96.9
SOD-1	4.5	6.0	89.5
Mean±SE	21.9 ± 11.1	4.9 ± 0.3	73.2 ± 10.9
	ABSOLUTE GENE DIVERSITY (Dm)		
	0.006±0.006	0.001±0.001	0.020±0.010
	Total (Dm) = 0.028±0.016		

Table 12. Absolute and relative gene diversity (Nei, 1975) at different taxonomic levels in European red deer. Possible hybrid populations or those with very small sample sizes ($n < 10$) were not included.

The relationship between subspecies was further explored by constructing both dendrograms and phylogenetic trees. Both procedures employ the pair group method, combining sites where the gene frequencies are most similiar, subsequently treating them as a single unit, and repeating the process until all sampling sites or groups of sites are incorporated. The dendrograms were constructed by calculating a genetic distance (Nei, 1972) from the gene frequencies of all loci, and clustering localities by the unweighted pair group method (UPGMA, Sneath and Sokal, 1973). The phylogenetic trees used a continuous maximum liklihood program (CONTML, Felsenstein, 1981) on polymorphic loci.

Examples of both types of topologies are shown in Appendix 4C. Although the structure of the diagrams differ and there are some differences in the relationship between particular localities, the general pattern of divergence is the same by both methods. C.e. elaphus populations cluster together, as do those of C.e. scoticus. The major branching is between these subspecies in both diagrams, with C.e. germanicus more closely associated with the former and C.e. atlanticus with the latter. The hybrid populations were more scattered, though most were positioned to C.e. elaphus. An exception to this in both the dendrogram and tree was Ankarsrum (site H). As this population was established from just three individuals, and only eleven deer were analysed, the pattern of relationship is more likely the result of a founder effect or sampling error than common descent with Scottish red deer. In order to eliminate the confounding effects of possible hybrid populations and those from which very few samples were obtained ($n < 10$) another dendrogram was constructed (Figure 12) eliminating these localities, though yielding the same general pattern.

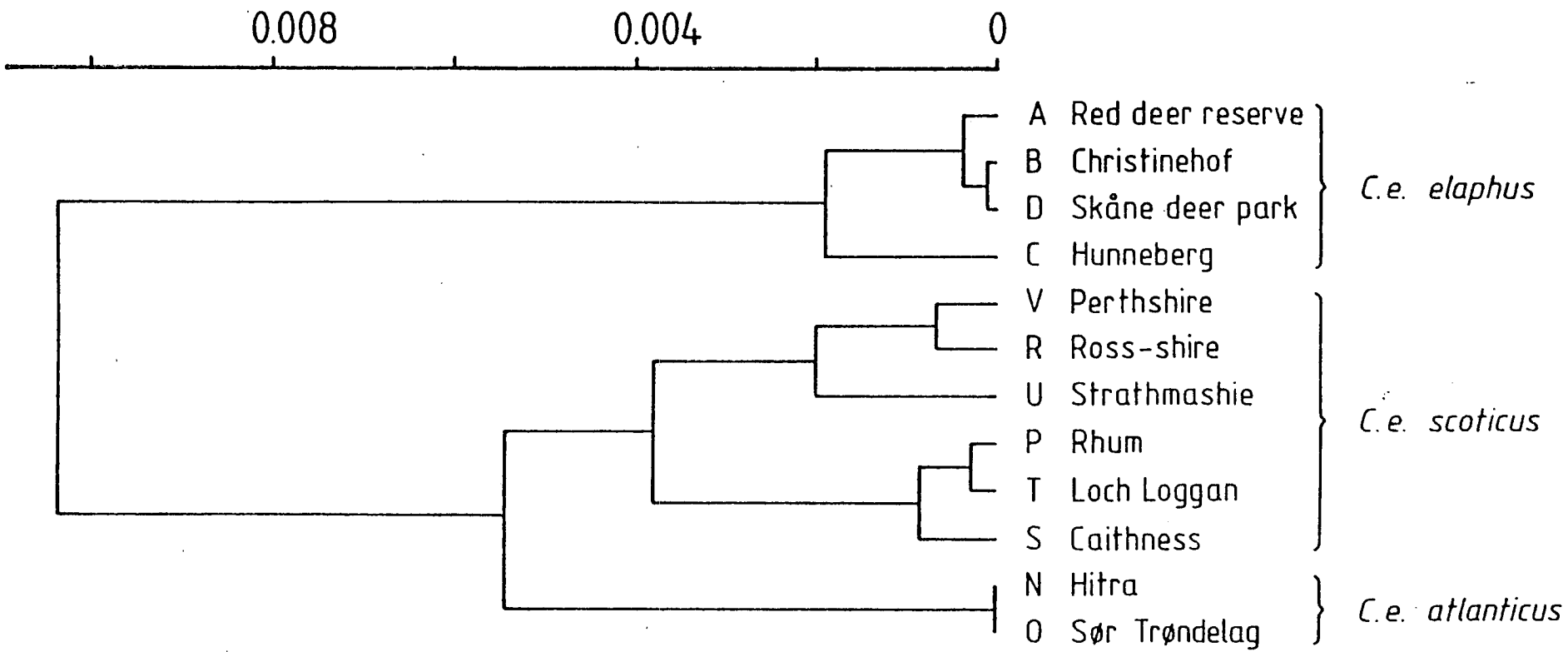


Figure 12. Dendrogram of genetic distance (Nei, 1972) of three European red deer subspecies.

The robustness of these topologies was tested in two different ways. Phylogenetic trees of this type are affected by the order in which populations were considered, so the gene frequencies from each site were altered until the tree with the highest log-normal likelihood came up repeatedly. The dendrogram was tested by selectively removing each polymorphic from the algorithm. Though each locus caused minor changes in the relationship of particular locations, only the removal of IDH-2 altered the major branching pattern between *elaphus* on the one side and *scoticus* and *atlanticus* on the other.

The reason for this is evident from the gene frequency data. At IDH-2, the slow allele predominates in all *C.e. elaphus* populations and is fixed in two of them, whereas the fast allele (IDH-2 125) predominates in all *C.e. scoticus* and is fixed for the two of *C.e. atlanticus*. Although rare alleles at other loci (GPI-1, PGM-1, PGM-2) contribute to the bifurcation between these groups, IDH-2 alone is diagnostic of this genetic differentiation.

TAXONOMIC UNIT	Groups	Loci	P	H
Red deer subspecies	4	34	0.077	2.2
Red deer populations				
C.e. elaphus	7	35	0.082	2.3
C.e. scoticus	6	34	0.138	3.0
C.e. atlanticus	2	35	0.000	0.0
C.e. germanicus	1	34	0.088	3.5
C.e. hybrids	6	35	0.081	2.8
Scandanavian moose	18	23	0.094	2.0
White-tailed deer	8	20	0.358	7.4
Fallow deer		25	0.000	0.0

Table 13. General indices of genetic variation comparing different taxonomic units of red deer and other cervid species.

The final comparison between subspecies was of the general

indices of variability (Table 13). Although the deer populations from Scotland and Norway were most closely related of the subspecies, the percentage of polymorphic loci and average heterozygosity were lowest in C.e. atlanticus (P = 0, H = 0), while those of C.e. scoticus (P = 0.013, H = 3.0%) were the highest. The average heterozygosity of Scottish red deer populations was significantly higher than the other populations (Mann-Whitney U- test, $p < 0.005$). Compared with the other two deer species in which several wild populations have been surveyed electrophoretically, European red deer showed an intermediate level of genetic variability.

4.3 Retesting red deer from Scotland, 1980

To check whether the genetic subdivison found initially among Scottish red deer was repeatable, as well as to try to test how that variation might be maintained, the electrophoretic survey was extended to thirteen populations (404 individuals) in 1980. Also, a visiting biologist brought 80 red deer samples from the Netherlands (C.e. hippelaphus) and these were analysed with the Scottish samples on the same starch gels.

In tripling the sample size, finite resources meant reducing accordingly the number of loci analysed. This was especially necessary as five of the six polymorphic loci in Scotland were dependent on the cofactor NADP for their enzymatic reaction, which is much more costly than NAD. Because part of the research project's purpose was to determine if electrophoretic analysis of culled red deer was applicable to management problems, where funds are often limited, it was considered appropriate to find out how much information could be gained by focusing on relatively few polymorphic

enzymes. Information on quantitative characters was collected for the deer sampled in 1980, and their correlation with isozyme variation is discussed in Chapter 5.

Eleven loci were tested in 1980, though the concentration was on three loci where polymorphism had been previously detected: IDH- 2, GPI-1 and SOD-1. The SOD-1 locus was scorable on the cathode portion of IDH stained gels. None of the other eight loci showed variation even with the increased sample size. The gene frequencies of polymorphic loci are listed in Table 14. Significant heterogeneity between populations was again observed, at all three polymorphic loci as well as for their cumulative effect. The relative genetic diversity (F_{st} = 11.4%) increased with more populations sampled, despite the reduction in the number of polymporphic loci analysed.

LOCATION	IDH-2	GPI-1	SOD-1	H%	P%
1. Caithness	2 19 12 0.348	28 1 0 0.983	26 7 0 0.894	6.1	27
2. Strathconon	0 8 32 0.100	36 1 0 0.986	17 19 4 0.662	6.0	27
3. Fairburn	0 7 12 0.184	16 3 0 0.921	19 0 0 1.0	4.0	18
4. Glen Fiddich	3 7 16 0.250	25 1 0 0.981	15 6 5* 0.692	7.6	27
5. Strathmashie (F.C.)	2 14 19 0.257	34 0 0 1.0	17 13 5 0.671	7.5	18
6. Loch Laggan	3 9 27 0.192	38 0 0 1.0	17 20 2 0.692	6.7	27
7. Rhum	6 24 8 0.474	30 9 0 0.885	38 1 0 0.987	6.6	27
8. Glen Tannar	1 11 11 0.283	20 3 0 0.935	12 8 3 0.696	8.6	27
9. Strathyre (F,C.)	5 35 24 0.352	59 3 0 0.976	41 19 4 0.789	7.6	27
10. Glen Artney	1 4 5 0.300	9 1 0 0.950	10 0 0 1.0	4.7	18
11. Glen Esk	1 7 16 0.375	23 1 0 0.979	12 7 5 0.646	8.8	27
12. Newton Stewart (F.C.)	3 19 5 0.608	32 5 0 0.932	36 0 0 1.0	5.5	18
13. Clattering-shaws (F.C.)	8 5 3 0.656	11 4 1 0.812	16 0 0 1.0	6.9	18
Chi-square contingency test d.f. p<	87.36 (12)***	32.91 (12)***	100.07 (12)***		220.34 (12)***
Fixation index				H_s	0.2444
H_t	0.4468	0.0964	0.2884	H_s H_t	0.2772
F_{st}	11.7%	5.5%	15.8%	Total F_{st}	11.83%

Table 14. The observed genotype and gene frequencies for three polymorphic loci sampled in 1980. General indices of variation are at the right. Heterogeneity test (X^2) and fixation indices (F_{st}) are at the bottom.

The fewer number of loci tested did inflate the other indices of genetic variability, and these cannot be considered typical of Scottish red deer. Both the relative and absolute genetic diversity of the Scottish populations was higher than that of those from the Netherlands. As with other Continental red deer populations tested the previous year, GPI-1 was polymorphic only in Scottish populations. The common allele at IDH-2, however, was the same in most Dutch and Scottish populations, so there was not the clear division as found between Scotland and Sweden the previous year. Moreover, the two Scottish populations sampled in Galloway had the same common allele as previously found in Sweden.

Interestingly, both measures of genetic distance indicated that the two Galloway localities were most closely related to deer from Rhum. All three of these present populations were the result of nineteenth century reintroductions, with some of the animals coming from English deer parks. Phylogenetic trees were constructed from the 1980 results (Appendix 4D). Because they are based on so few polymorphic loci, however, the confidence intervals of estimated branch lengths overlap, so these relationships must be considered tentative.

Chapter 5

ENZYME POLYMORPHISM, ADAPTATION and QUANTITATIVE TRAITS

> ... Variations, however slight and from whatever cause proceeding, if they be in any way profitable to a species, in their infinitely complex relations to other organic beings and to their physical conditions of life, will tend to the preservation of such individuals, and will generally be inherited by their offspring.
>
> Charles Darwin
> On the Origin of Species

5.1 Neutrality vs. selection hypotheses

In addition to testing whether the genetic subdivision found in red deer populations was repeatable, in the second year of the study an attempt was made to determine whether natural selection was acting on the electrophoretic variants detected. Generally, random genetic drift and inbreeding promote subpopulation divergence, whereas migration thwarts it. Selection, as mentioned earlier, could act in either direction, and thus cannot be ignored in a study which uses genetic variation to characterize population structure.

Though evidence of selection on multi-genic phenotypic traits abounds in natural populations, the case for selection on specific electrophoretic variants is much less compelling. For this reason, combined with the ever-increasing amount of protein variation detected (Jones, 1980), it has been hypothesized that isozymes are selectively neutral (Kimura, 1968). Because the problem is crucial for the application of electrophoresis to evolutionary questions, it has been vigorously debated (Lewontin, 1974 and Ayala, 1976 offer discussions) and much less successfully tested; Hartl (1980) describes several

approaches yielding ambiguous results.

The greatest obstacle to testing the selection and neutrality hypotheses is in associating fitness values to characteristics which can be recognized and quantified in individuals or populations. The problem is that fitness is a phenotypic measurement, albeit a complex one, yet it is the genetic variation for fitness that is important on an evolutionary scale. On the other hand, while there is considerable understanding of the genetic behaviour of allozymes, their phenotypic effects remain largely unknown. Falconer (1983, p.301) addressed this problem by delineating the components of fitness and outlining how genetic correlations with those components could be tested. Two approaches were used in this study to test selection in red deer, the first focusing on populations and habitat types, the second on correlation with quantitative characters of individuals.

5.2 Local adaptation

If selection is acting at the molecular level and particular isozymes are contributing to local adaptation, this should be most readily observable where environments are most different. The converse of this idea is testable, by grouping similar environments. If such a grouping accounts for more of the between-location variation, then again selection is implicated. The difficulty arises in choosing which environments are most similar, in the "infinitely complex relations" between red deer and habitat.

The primary criterion used in grouping Scottish red deer habitats was tree cover. Red deer are primarily an animal of the forest edge: Staines (1976) found that shelter was a limiting factor in the winter movement of deer in the northest Highlands, and Grace and Easterbee

(1979) calculated that there would be nearly twice the heatloss in deer on open hillside than in woodland. To test whether this factor affected isozyme frequencies, wherever possible open hill habitats were sampled with neighboring forested land in 1980 (Figure 2, Chapter 2).

Nested gene diversity analysis (Charkraborty et al., 1982) was employed to evaluate levels of organization hierarchically, as used previously to compare red deer populations across northern Europe. In this case, however, habitat type was substituted for geographic or taxonomic units. The relative genetic diversities (G_{st}) at each level are listed below, with standard errors in parentheses.

INDIVIDUALS	LOCALITIES	HABITATS
87.9% (0.9%)	10.6% (1.6%)	1.5% (0.9%)
		RANDOM
87.9% (0.9%)	11.0% (1.4%)	1.1% (0.6%)

The results show that open hill vs. forested habitats account for very little of the gene frequency differences between localities; the difference between using this environmental criterion and grouping the localities randomly into two classes was insignificant. Thus isozyme variants for GPI-1, IDH-2 or SOD-1 in red deer do not appear selected for open hill or forested habitats.

When localities were grouped by latitude and longitude, more of the relative genetic diversity was accounted for: 6.8% ($\pm$1.5%) and 7.0% ($\pm$0.6%) respectively. However, these tests were confounded by sampling paired localities for the previous habitat selection analysis. Since the paired localities were classed in the same latitude and longitude, the results could as readily be attributable to inbreeding or genetic drift as the environmental effects they sought to measure. This drawback seems inherent to field studies

attempting directly to measure selective effects in diverse environments; in trying to isolate one effect, others will usually be biased if the same localities are used. The focus of the selection experiments was therefore shifted to individual deer, testing the correlation of genotypes with characters which might be causally related to reproductive success.

5.3 Correlation with quantitative characters

To test the neutrality hypothesis for electrophoretic variants in individuals, data on the sex, age and gralloch weight was collected for deer sampled in 1980, as well as the number of antler points in stags and the lactation status of hinds. Their genotypes at GPI-1, IDH-2 and SOD-1 were recorded, and the percentage of heterozygotes in each individual was subsequently calculated. Individual heterozygosity has been associated with components of fitness, from aggressive and exploratory behaviour in mice (Garten, 1976, 1977) to growth rates in oysters (Singh and Zouros, 1978) and productivity in deer (Johns et al., 1977). Correlations between the quantitative characters and genotypes or heterozygosity were evaluated by two-way analysis of variance, using the least squares program developed by Harvey (1960) and modified in 1980 by the Animal Breeding Research Organization.

The relationship between the quantitative traits recorded and components of fitness is not known in most large non-domestic vertebrates, but since the pioneering ethological work of Darling (1937), red deer in Britain are among the most extensively studied of large mammals, and fortunately some of the most recent research focuses on lifetime reproductive success (Clutton-Brock et al., 1982,

pp. 152-156). Unfortunately, the data obtained for these carcasses, though related to immediate fitness, are but a few of many factors that contribute to lifetime reproductive success.

In Glen Feshie hinds, for example, Mitchell and Brown (1974) found that fertility was primarily related to carcass weight and age, and secondarily to lactation status - having a calf at foot reduced the probability of breeding. It is generally recognized that hinds living on farms or in forests have higher pregnancy rates than those on the open hill. Fecundity, a hind's ability to both conceive and carry a calf to parturition, is however only one element of reproductive success. On the Isle of Rhum, calf survival accounted for 75.1% of the variance in hind reproductive success, whereas fecundity accounted for only 20.7% (Clutton-Brock et al., 1982, p.83). Calf survival may be more important on Rhum, where both weather conditions are harsh and deer density is high in the absence of culling, but the results point out the complexities incurred in measuring lifetime reproductive success indirectly.

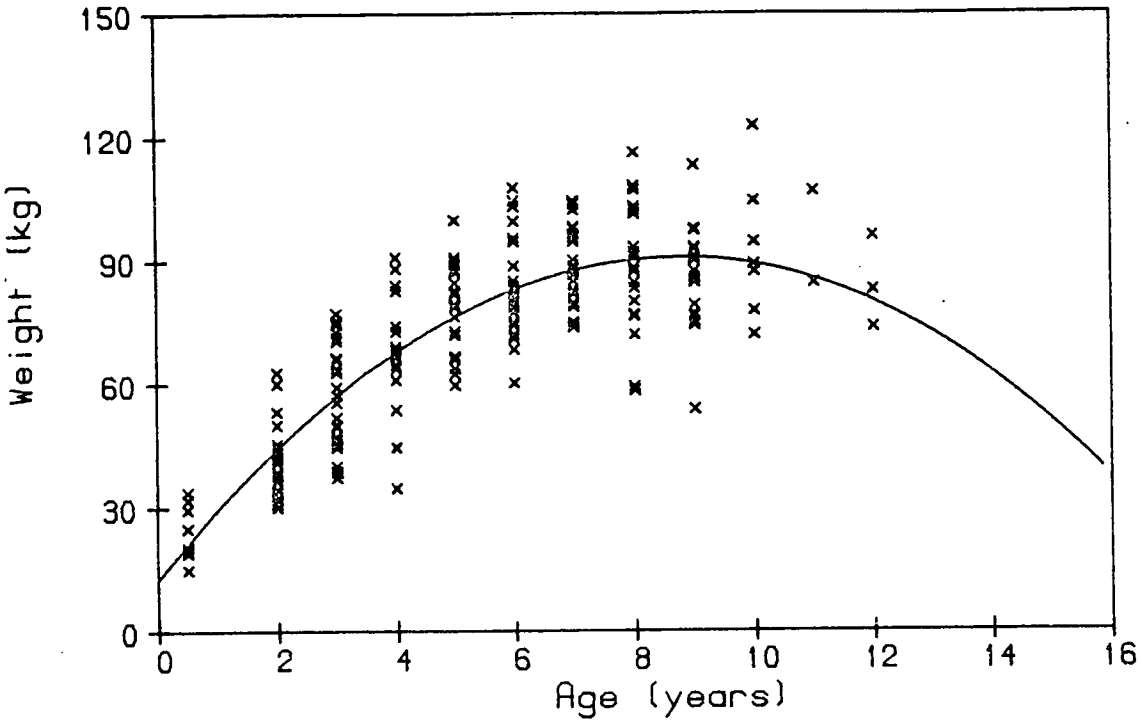
Nonetheless, heterozygosity did not significantly affect either hind weight ($F=2.05$ d.f.$=3$ $p>0.1$) or lactation status ($F=0.95$ d.f.$=3$ $p>0.4$), nor did any enzyme locus (detailed analysis of variance results are compiled in Appendix 5). The factors which did affect weight and lactation status were location and age. Both the linear and quadratic regression of age were highly significant ($p<0.001$), indicating that lighter weight and lower fecundity characterize both young and old hinds. This supports the observations both on Rhum and Glen Feshie, where few hinds bred successfully before age 3 or after age 10.

In stags, holding hinds by fighting or intimidating opponents is

crucial to reproductive success, and this in turn is related to body weight and condition (Clutton-Brock et al., 1982). However, as harem-holders eat seldom during the rut, probably due to both vigilance and hormonal changes (Kay, 1978), stags can lose up to 20% of their body weight during that time (Mitchell et al., 1976). Because the shooting season overlaps with the rut, stag weight thus would not be an adequate reflection of breeding success without the date the animal was shot. Noting this limitation of the data, weight was not significantly correlated with either particular isozymes or overall heterozygosity. As with hinds, the effect of age and locality on stag weight was highly significant ($p<0.001$). The relationship between weight and age for both hinds and stags is shown graphically in Figure 13.

The only significant effect found for overall heterozygosity was on the number of antler points ($F=3.34$ d.f.$=2$ $p<0.05$). The most heterozygous stags had the most antler points (independent of age and location), though stags with no heterozygotes had more points than those which were heterozygous at only one locus, and the standard errors of all three classes overlapped (detailed in Appendix 5). Antler point number, though probably a more crude gauge of reproductive success, is not subject to the bias of weight loss on successful rutters (stags which lose part of their antlers also tend to lose fights). Also, antler points are a selective criteria for many stalkers who prefer to let some stags with "good heads" live and breed, while shooting hummels, switches and animals which are "going back", i.e. losing points with advanced age.

The only significant single locus effect found was on age. As none of these deer died of natural causes, age should more properly be



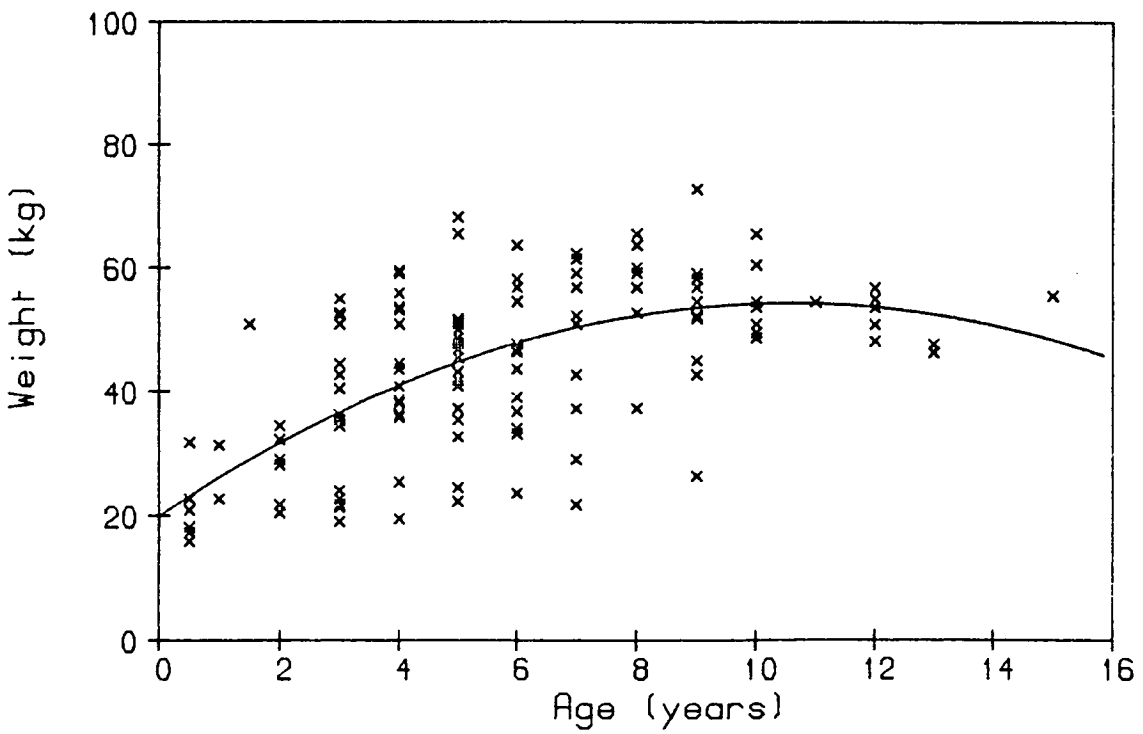


Figure 13. Regression of weight on age in stags (above) and hinds sampled throughout Scotland in 1981.

called survivorship - the ability to survive successive culls. There was a significant relationship between SOD-1 genotypes and survivorship ($F=4.55$ d.f.=2 $p<0.02$) and again it was the heterozygotes at SOD-1 which, of the three genotypes, lived the longest. As mentioned in Chapter 3, the function of this enzyme is to remove oxygen-free radicals from the blood, and the accumulation of these radicals has been associated with aging (Comfort, 1978, p. 276). However, it is unknown how being a heterozygous SOD helped deer of both sexes to survive longer. The effect of locality on survivorship was highly significant ($F=9.36$ d.f.=15 $p<0.001$); deer on Forestry Commission sites were shot an average of two years earlier than those at the other nine localities.

There is little evidence of correlation between the isozyme variants scored in the lab and the quantitative traits measured by stalkers in these red deer. As it was heterozygotes which were favoured in the two cases when significant effects were found, these could as likely be the result of heterosis - particularly as population subdivision has been demonstrated- as any selective benefit conferred by these isozymes.

In contrast to the isozymes, locality had a highly significant effect on the quantitative characters, as predicted by Mutch et al. (1976): "When the animal we see on the hill is so much a fortuitous product of its particular part of the range, selection by appearance (phenotype) cannot be very effective."

This idea was pursued by repeating some of the analyses, treating locality as a fixed rather than a random effect so that differences in quantitative characters at particular locations could be assessed. Looking at weight independent of age, for example, Rhum stags were the

lightest of those tested (28.6 ±5.1 lbs. below the mean) and Glen Tanner stags the heaviest (33.4 ±8.1 lbs above the mean). The culling season bias should, if anything, favour heavier Rhum stags as most were shot before or early in the rut; the author accompanied the stalkers during the latter stages of the cull. Rhum is among the most exposed of the localities tested and Glen Tanner still has a large contiguous pinewood; moreover, Glen Esk, adjoining Glen Tanner to the south, but lacking cover, had stags averaging 4.6 (± 7.3 lbs.) below the mean. Although tree cover does not exert selective pressure on the isozymes, it does appear to affect stag body weights.

Since locality had such a significant effect on phenotypic characters, and differences in gene frequency across localities had been found, the final analysis of variance was of locality on heterozygosity. However, neither location ($F=1.04$ d.f.=15 $p>0.4$) nor sex ($F=0.87$ d.f.=1 $p>0.3$) had a significant effect on heterozygosity in the 1980 red deer samples.

Chapter 6

ELECTROPHORETIC VARIATION IN WAPITI

6.1 Why wapiti were tested

In contrast to the considerable electrophoretic variation detected in most European red deer populations, the general indices of genetic variation were low in the only published isozyme study of North American wapiti (P = 0.041, H = 1.2%, Cameron and Vyse, 1978). As mentioned earlier, it is generally recognized that red deer and wapiti are closely related, if not the same, species.

Assuming that the electrophoretic methods were similar in both studies (and there was good reason to expect the latter as I learned electrophoresis from Drs. Cameron and Vyse), several hypotheses might explain the low level of variation previously reported.

1) The wapiti samples could have denatured, either before they were collected or before electrophoretic analysis, and allelic variants for less stable enzymes could have gone undetected.

2) North American wapiti could have generally lower electrophoretic variation than red deer, perhaps as a result of a founder effect in the Pleistocene radiation of wapiti over the Bering Land Bridge. If the low variation was consistent for wapiti throughout their North American range, the genetic distance between red deer and wapiti would likely be larger than that between *Cervus elaphus* subspecies in Europe.

3) The Yellowstone population which was sampled might not typify North American wapiti. Just as the more than 100 red deer sampled

in Norway were monomorphic at all 34 enzyme loci analysed, there could be significant genetic differences between wapiti populations, particularly those from different geographic areas or representing different recognized subspecies.

To test these hypotheses, 253 wapiti samples were collected from thirteen localities in the northwest United States. They were run on the same electrophoretic gels with 66 red deer from Scotland, to confirm the common migration rates of shared alleles. Fifty of the Scottish sample were collected from six localities in 1981, within a month of travelling to the United States. The remaining sixteen, which had been collected and analysed in 1980, were included to test the effects of extended freezing on isozyme denaturation.

The denaturation experiment was enhanced by the United States Department of Agriculture Animal and Plant Health Inspection Service. The red deer samples were held in the freezer compartment of a conventional refrigerator (approximately $0^{o}C$) for four months until a ban on meat imports from the U.K. to the U.S. was lifted in January, 1982. Of 48 trials (16 samples at three polymorphic loci), three samples scored differently and five were unscorable after sixteen months. Mercapto ethenol added to the homogenates improved the resolution of some samples. Banding patterns from kidney homogenates had better resolution after storage than those from muscle, particularly at IDH-2 and SOD-1, probably due to higher concentration of the enzymes. The percentage of unscorable samples for each of the three groups are listed below.

	Scorable	Unscorable	%
1980 Red deer	43	5	10.4
1981 Red deer	145	5	3.3
1981 Wapiti	654	15	2.2

Table 15. Percentage of samples which were unscorable at three enzyme loci (GPI-1, IDH-2, SOD-1) after eighteen, five and three months of freezing.

Though the trend was for a higher percentage of scorable loci in more recently collected samples, neither the difference between red deer of different years (X^2=3.80 d.f.=1 $p>0.05$) nor between red deer and wapiti (X^2=3.77 d.f.=1 $p>0.05$) was significant. There was a significant difference between the 1980 red deer and the wapiti samples (X^2=11.03 d.f.=1 $p<0.001$). Unless the Yellowstone wapiti sample denatured prior to freezing as a result of carcass overheating, it seems unlikely that the low heterozygosity found in that wapiti population was an experimental artifact.

6.2 Heterogeneity in Northwest wapiti populations, 1981

Wapiti were analysed for most of the same enzyme systems tested previously in red deer. Six loci were omitted (EST-1,2,3, MDH-1,2 and ME) as the banding patterns could not be read clearly in many samples. In contrast to the single polymorphic locus found previously in Yellowstone wapiti, 5 of the 28 enzyme loci which were analysed from Northwest populations showed allelic variation (Table 16). Two of these, MPI and PEP-2, were not polymorphic in the red deer run on the same starch gels.

Mannose phosphate isomerase (MPI) was the only locus which was polymorphic at all wapiti localities. IDH-2 was polymorphic at all sites except the Olympic Peninsula, Washington, whereas SOD-1 was monomorphic at Mt. St. Helens, Washington and two of the four Idaho

localities. For both PGM-1 and PEP-2, wapiti sampling sites west of the Cascade Mountains generally showed variation and those east of the mountains did not. The Cascade crest has historically been considered the boundary between the Rocky Mountain and Roosevelt subspecies of wapiti, though the Boone and Crocket Club, which registers trophy animals, has recently made its boundary Interstate Highway 5, west of the mountains.

LOCATION	LOCUS				
	IDH-2	MPI	PEP-2	PGM-1	SOD-1
Yakima, WA	0.083 (6)	0.929 (7)	1.0 (7)	1.0 (7)	0.071 (7)
Mt. Ranier, WA	0.031 (16)	0.967 (14)	1.0 (12)	0.969 (16)	0.036 (14)
Mt. St Helens, WA	0.180 (25)	0.839 (28)	0.912 (17)	0.964 (28)	1.0 (15)
Olympic, WA	0.0 (24)	0.913 (23)	0.905 (21)	0.813 (24)	0.383 (24)
Pacific, WA	0.192 (13)	0.808 (13)	0.958 (12)	0.917 (12)	0.269 (13)
N. Ukiah, OR	0.250 (6)	0.917 (6)	1.0 (6)	1.0 (6)	0.250 (6)
S. Ukiah, OR	0.250 (10)	0.938 (8)	0.900 (10)	0.944 (10)	0.100 (10)
Region 4, ID	0.100 (30)	0.855 (31)	1.0 (30)	1.0 (30)	0.0 (31)
Region 6, ID	0.040 (62)	0.847 (62)	1.0 (62)	1.0 (61)	0.048Y (62)
Region 7, ID	0.029 (34)	0.843 (35)	1.0 (35)	1.0 (34)	0.014 (35)
Region 9, ID	0.167 (9)	0.944 (9)	1.0 (9)	1.0 (9)	0.0 (9)
Chi-square contingency test (d.f.)	30.24 (10)***	7.76 (10)ns	29.74 (10)***	49.51 (10)***	81.66 (10)***
Log-likelihood ratio test	29.67	8.99	27.42	39.00	71.75
F_{st}	7.2%	2.6%	6.0%	7.4%	16.8%

Table 16. Gene frequencies for five polymorphic loci in Northwest wapiti. Number of samples tested at locus are in parentheses for each population. F_{st} is the relative genetic diversity for each locus. Asterisks denote level of significance.

As with the Scottish populations the previous year, no wapiti loci significantly deviated from Hardy-Weinberg expectations. Also as in red deer, the gene frequency data indicated highly significant heterogeneity between populations - at four of the five polymorphic

loci (Table 16) and cumulatively (X^2=198.91 d.f.=50 $p<0.001$). The same level of significance was found using a log-likelihood ratio test (G=176.83 d.f.=50 $p<0.001$). Ten percent of the variation detected is due to differences between rather than within populations (H_s = 0.132 H_t = 0.147 F_{st} = 10.2%).

The general indices of genetic variation (Table 17) suggest the source of differences between this wapiti survey and the previous study. The number of polymorphic loci varied between two (Idaho Regions 4 and 9) and five (Pacific County, Washington). The percentage of polymorphic loci was significantly higher in Washington localities than those from Idaho (Mann-Whitney U test, n1=5, n2=4 U = 19 $0.025< p <0.05$). MPI and IDH-2 were the only loci which were consistently polymorphic throughout Idaho. Cameron and Vyse (1978) found variation at the former in Yellowstone wapiti, and did not test the latter. Recent work by Baccus et al. (1983) confirm wapiti MPI variation in Montana, which neighbors Idaho and Yellowstone.

LOCATION	P	H(%)	H_s	
Yakima, WA	0.107	1,49	0.083	
Mt. Ranier, WA	0.143	1.12	0.051	
Mt. St. Helens, WA	0.143	2.84	0.159	
Pacific County, WA	0.178	4.45	0.249	
Olympic Peninsula, WA	0.143	3.91	0.222	
North Ukiah, OR	0.107	3.22	0.180	
South Ukiah, OR	0.178	3.42	0.191	
Region 4, ID	0.071	1.53	0.086	
Region 6, ID	0.107	1.53	0.085	
Region 7, ID	0.107	1.25	0.070	
Region 9, ID	0.071	1.37	0.077	
	0.124	2.34	0.132	H_s
			0.147	H^s
			10.22%	F^t_{st}

Table 17. The percentage of polymorphic loci (P) and average heterozygosity including and excluding monomorphic loci (H and H_s) for thirteen wapiti locations in three states. F_{st} is the relative genetic diversity for the five polymorphic loci.

Comparison of average heterozygosity (H) between populations is more instructive, considering the history of western Washington wapiti. The difference in H values is not significant between Washington and Idaho (Mann-Whitney U = 5 $p > 0.1$) or in other comparisons between states. However, wapiti from two of the Washington localities sampled have grown out of translocations from Yellowstone, as have the Idaho wapiti (Bryant and Maser, 1982). If Mt. Ranier and Yakima wapiti are grouped with those from Idaho and compared with the remaining western Washington sites, the difference in heterozygosity is significant (Mann-Whitney U = 18 $p < 0.025$). Thus, it appears that the low average heterozygosity which Cameron and Vyse reported in the Yellowstone National Park population, though not typical of all wapiti, still characterizes animals introduced from Yellowstone fifty years ago.

Why do the Mt. Ranier wapiti not also have a lower percentage of

polymorphic loci than other populations around Puget Sound? The lower heterozygosity at Mt. Ranier is not the result of a few polymorphic loci, but of rare alleles at the loci which are polymorphic (see Table 16). There were recorded sightings of wapiti prior to the translocation from Yellowstone (National Park Research Biologist James Agee, pers. com.). Perhaps the current Mt. Ranier population has been built from those introduced animals and the small number of endemic wapiti which remained, with the consequent loss of heterozygosity.

6.3 Comparison with red deer

Just as two of the isozyme variants found in 1981 were unique to wapiti, one (GPI-1) was polymorphic only in red deer. At PGM-1 rare alleles occurred in both red deer and wapiti populations, whereas PGM-2 was polymorphic in neither. The fast allele of isocitrate dehydrogenase (IDH-2, 125) was the more common in the Scottish red deer populations ($p = 0.656$) as it had been in the two previous years. This allele was even more predominant in the Northwest wapiti ($p = 0.883$); it was fixed on the Olympic Peninsula, as it had been in Norway.

Superoxide dismutase (SOD-1) was the most diagnostic locus in differentiating wapiti and red deer. At this cathodal locus (in acid citrate buffer), the slower (SOD-1, -100) allele predominated in red deer populations ($p = 0.875$) as it had in all European red deer populations tested previously. The faster allele (SOD-1, -225) was much more common in all Northwest wapiti populations, with the gene frequency of nearly ninety percent ($p = 0.102$). Sampling fewer animals, Baccus *et al.* (1983) found a comparable though even more dramatic difference at SOD-2: one allele was fixed for wapiti, the

other for red deer. The contribution of each locus to the difference between red deer and wapiti is shown is the hierarchical gene diversity analysis (Chakraborty et al., 1982) below.

LOCUS	INDIVIDUALS	LOCATIONS	RED DEER/WAPITI
IDH-2	0.849	0.081	0.070
MP1	0.937	0.026	0.037
SOD-1	0.405	0.065	0.530
PGM-1	0.899	0.086	0.014
PEP-2	0.931	0.059	0.009
GPI-1	0.198	0.650	0.152
	62.7%	12.7%	24.6%
	0.627 (0.134)	0.127 (0.068)	0.246 (0.136)
	0.026 (0.012)	0.005 (0.003)	0.010 (0.008)

Table 18. Relative gene diversity (G_{st}) evaluated at each polymorphic locus. Below the line, the total relative and absolute gene diversities, with standard errors in parentheses.

As is apparent from the table, SOD-1 is responsible for most of the divergence detected between red deer and wapiti. Although MPI, PEP-2 and GPI-1 variants characterize one group or the other, because the alternate alleles generally have low frequencies, they contribute much less than SOD-1 which has different predominant alleles for the two groups.

The overall relative genetic diversity changed markedly when red deer and wapiti were considered together. The amount of variation attributable to populations was only 8.5% in wapiti - similar to that found in previous years in Scottish red deer. When red deer and wapiti were tested together, the source of non- individual variation totals 37.3%. Nearly twice as much of this variation is due to differences between red deer and wapiti (24.6%), than due to differences between populations within the two groups (12.7%), though the large standard errors decrease the significance of this gap. The absolute genetic diversity increases only slightly when red deer are

added (from 0.026 to 0.041), with only one more polymorphic locus at two of five red deer localities.

The number of red deer samples brought for direct comparison was quite small, and virtually all stags, a necessary consequence of leaving in the middle of the shooting season to organize wapiti sample collection in November of the same year. Moreover, the reintroduced Galloway red deer made up a larger proportion of the 1981 samples than previously, resulting in a much higher relative gene diversity between Scottish populations (G_{st} = 27.9%) than in the surveys of the two previous years. To check whether the division between red deer and wapiti still held with a more representative Scottish red deer sample, the gene diversity analysis was repeated substituting the 1979 Scottish population gene frequencies at the same 28 loci.

INDIVIDUALS	LOCATIONS	RED DEER/WAPITI
67.9%	5.8%	26.3%
0.679 (0.15)	0.058 (0.01)	0.263 (0.15)
0.026 (0.01)	0.002 (0.001)	0.010 (0.009)

Table 19. Relative and absolute gene diversity combining Scottish red deer (1979) and Northwest wapiti (1981) samples.

The pattern of gene diversity attributable to different levels of organization thus persist when more Scottish red deer, mostly from different localities, are analysed with wapiti. The total absolute gene diversity changed only slightly (reduced from 0.041 to 0.038) is still only a small percentage of the genome tested. Looking at the relative genetic diversity, the proportion of variation due to individual differences increased (from 62.7% to 67.9%) as did that between wapiti and red deer (from 24.6% to 26.3%). The reduction in variation between locations (from 12.7% to 5.8%) is not unexpected, as the reintroduced Galloway population was not sampled in 1979. It

results in a significant division between the variation attributable to local populations and that due to red deer-wapiti differences.

The division between red deer and wapiti is also reflected in the dendrogram of Nei's (1972) genetic distances (Figure 14). The distance separating these groups is an order of magnitude greater than that separating localities within Scottish red deer or Northwest wapiti. Moreover, this bifurcation is derived from the total wapiti and red deer sample (n = 253 and 153 respectively), so is less subject to sampling error than locality relationships. An even larger divergence was determined independently (Baccus et al., 1983) running Swedish red deer and Montana wapiti on the same gels.

Several of the relationships between wapiti localities in the dendrogram tempt speculation. Mt. Ranier and Yakima, the most closely related of localities, were both sites of translocations from Yellowstone National Park, which was also the source of much of the present Idaho population. The Mt. St. Helens population may also have received Yellowstone wapiti, and the instability of the mountain may have resulted in several periods of immigration. It is estimated that 2000 wapiti were killed in the 1980 eruption, and a population on the devastated northeast side of the volcano has already become re-established (personal observation, August 1982; Evelyn Merrill, pers. com.). Pacific County, WA. borders Oregon, though is not particularly close to the Ukiah, OR. sampling localities. Finally, the Olympic Peninsula wapiti, which are the most genetically distinct of the localities sampled, is the type locality of the Roosevelt wapiti subspecies. There are no recorded transplants to the peninsula, and immigration from the east is blocked by Puget Sound.

However, any conclusions about these relationships must be

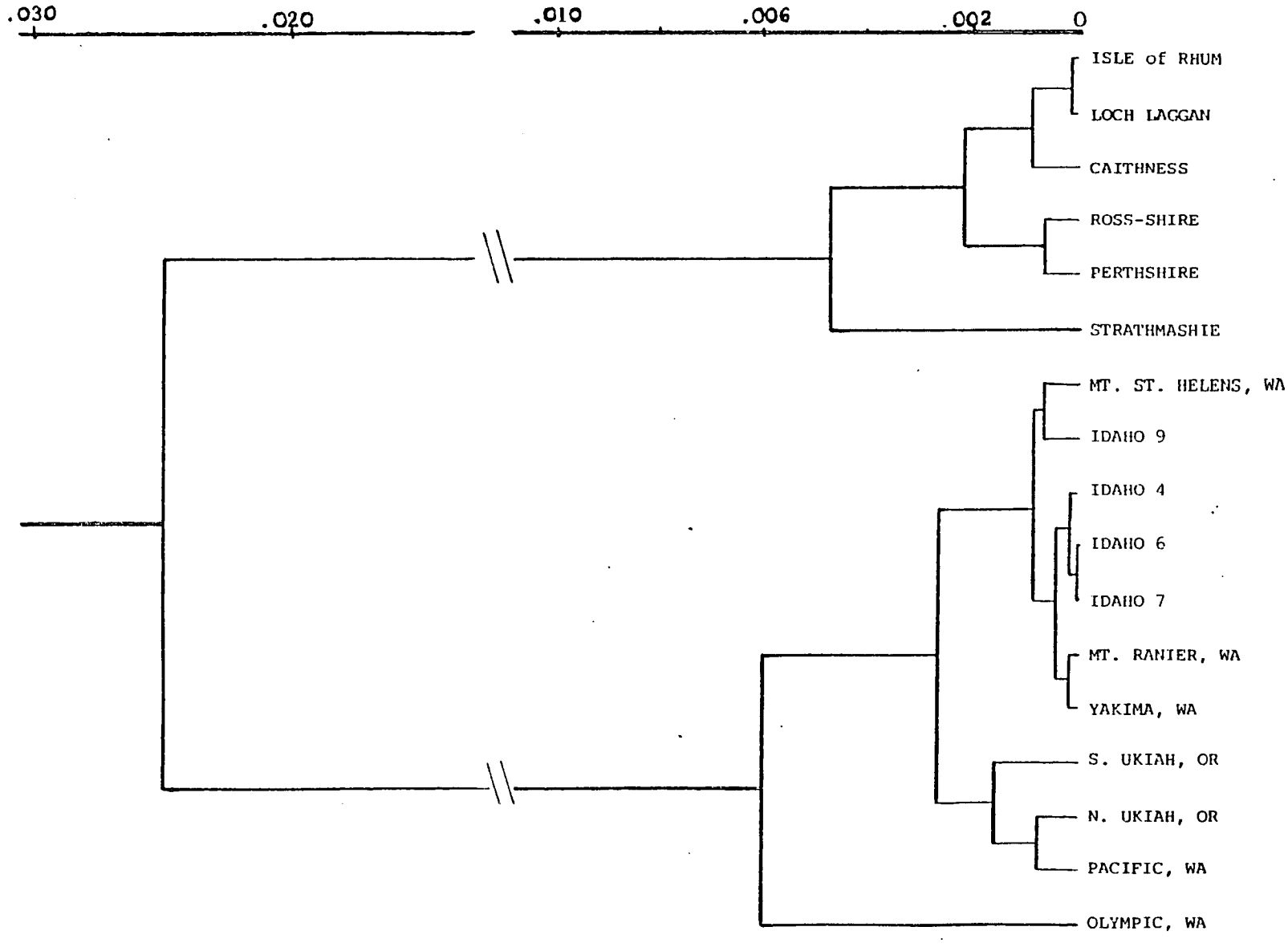


Figure 14. Dendrogram of genetic distance (Nei, 1972) between populations of Scottish red deer and North American wapiti.

tempered by looking at the red deer section of the topology, and recalling that the Strathmashie (n = 14) divergence was not repeatable in 1980 when the sample size was increased and the number of polymorphic loci tested was reduced. Sample sizes from several of the Northwest locations are the same or less than that from Strathmashie (Table 16). The goal of the 1981 wapiti study was to survey as many different populations as possible rather than many individuals in a single population, as had been done previously. Conclusions about the genetic relationship between localities in such close proximity (both in geographic and genetic distance) will require substantiation, analysing more individuals at more loci, perhaps over several years.

The final gene diversity analysis incorporated continental European red deer populations as well, and the levels of organization were treated first geographically and then according to current taxonomy. In the geographic comparison, all localities with a sample size greater than ten, which had been tested electrophoretically at the same 28 loci, were included. In the taxonomic comparison, the same minimum sample size was imposed, and animals of known hybrid origin were omitted. One exception was allowed in both cases: although there were only nine samples from Nationalpark Bayerische Wald, because it was the only locality from Germany and the only representative of the Cervus elaphus germanicus subspecies it was included.

INDIVIDUALS	LOCALITIES	STATES,COUNTRIES	CONTINENTS
625	28	7	2
54.8% (0.09)	6.0% (0.01)	9.5% (0.06)	29.7% (0.14)
0.022	0.002	0.003	0.012
INDIVIDUALS	POPULATIONS	SUBSPECIES	RED DEER/WAPITI
551	23	6	2
54.2% (0.09)	3.5% (0.003)	11.6% (0.07)	30.6% (0.14)
0.022	0.001	0.005	0.013

Table 20. Genetic diversity of European red deer and North American wapiti over 28 enzyme loci (8 polymorphic). Beneath the number comprising each level of organization is the relative genic diversity with the associated standard error in parentheses. The bottom row is the absolute diversity - including monomorphic loci.

Whether broken down geographically or taxonomically, the proportion of variation attributable to different levels of organization is very similar. The addition of continental deer further increased the percentage of variation accounted for by the red deer/wapiti division. Present subspecies account for slightly more of the variation than do states and countries, but the comparison is not a direct one, as hybrids were not included in the taxonomic comparison. If the red deer/wapiti distinction is removed in the taxonomic analysis (i.e. Cervus canadensis is discarded, as is the trend) all of that variation falls to the next lower level of organization, and 42.3% ($\pm$ 0.09) is attributable to subspecies.

The absolute genetic diversity in both geographic and taxonomic breakdowns totals 0.041 ($\pm$ 0.02). This small absolute figure, less than 5% of the genome, is understandable when considering that 20 of the 28 loci tested were monomorphic, and half of the polymorphic loci (MDH-1, PGM-1, PGM-2, PEP-2) had quite rare allelic variants, and only at a few of the localities. Although the red deer and wapiti which were sampled do have largely a common genome (at least as it was surveyed here by starch gel electrophoresis), the variation which was detected does substantiate the relationship between different groups.

This is reflected in the final phylogenetic tree, which used the same data as the taxonomic gene diversity analysis (Figure 15). The major separation is between red deer and wapiti. Within the wapiti line, the Olympic Peninsula population is most divergent. The Scottish red deer all group together as do the Swedish red deer. The deer from Norway are the most distant branch of the Scottish line, as is the German locality from Sweden. The confidence intervals for most of the branch lengths are large and are a result of the limited number of polymorphic loci used to compare such closely related animals.

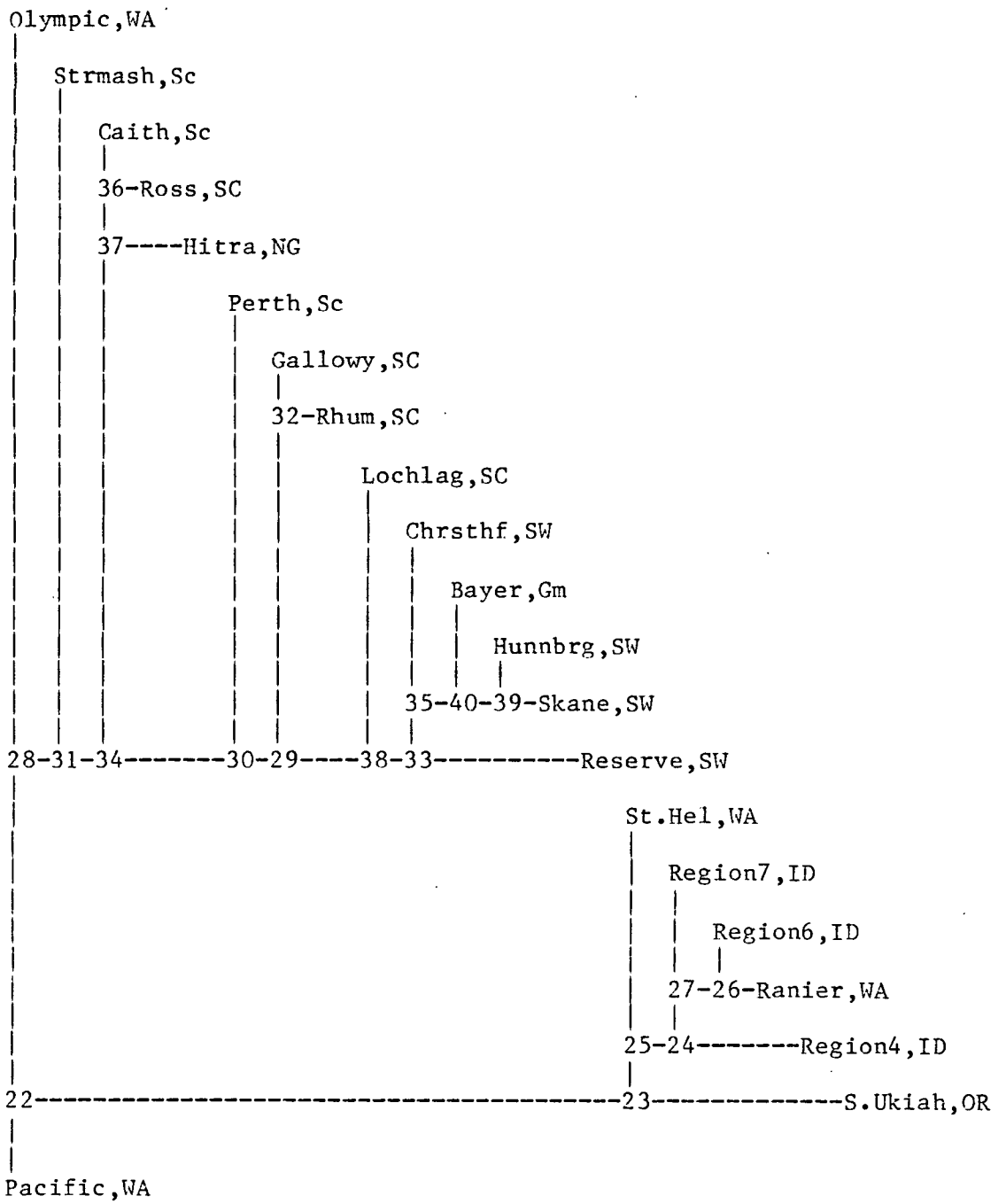


Figure 15a. An unrooted phylogenetic tree of European red deer and North American wapiti constructed according to Felsenstein (1981).

Between	And	Length	Approx. Confidence Limits	
Pacific,WA	22	0.00000	(-0.00687,	0.01120)
22	28	0.01108	(-0.00990,	0.04526)
28	Olympic,WA	0.02391	(-0.00051,	0.06371)
28	31	0.06694	(0.01248,	0.15571)
31	Strmash,Sc	0.00000	(-0.00735,	0.01199)
31	34	0.01099	(0.00249,	0.02484)
34	37	0.00901	(0.00195,	0.02053)
37	36	0.00000	(-0.00580,	0.00945)
36	Caith,Sc	0.02608	(0.00702,	0.05714)
36	Ross,SC	0.00000	(-0.00444,	0.00724)
37	Hitra,NG	0.04417	(0.01188,	0.09679)
34	30	0.00069	(-0.00343,	0.00741)
30	Perth,Sc	0.00000	(-0.00196,	0.00319)
30	29	0.00438	(0.00065,	0.01044)
29	32	0.00060	(-0.00126,	0.00363)
32	Gallowy,SC	0.00166	(0.00011,	0.00419)
32	Rhum,SC	0.00060	(-0.00052,	0.00241)
29	38	0.00230	(0.00001,	0.00603)
38	Lochlag,SC	0.00000	(-0.00204,	0.00332)
38	33	0.02438	(0.00614,	0.05409)
33	35	0.00104	(-0.00060,	0.00372)
35	Chrsthf,SW	0.00000	(-0.00126,	0.00206)
35	40	0.00690	(0.00136,	0.01593)
40	Bayer,Gm	0.02862	(0.00724,	0.06347)
40	39	0.00069	(-0.00388,	0.00814)
39	Hunnbrg,SW	0.02357	(0.00634,	0.05164)
39	Skane,SW	0.00000	(-0.00361,	0.00589)
33	Reserve,SW	0.00126	(-0.00039,	0.00395)
22	23	0.01398	(0.00376,	0.03063)
23	25	0.01745	(0.00470,	0.03824)
25	St.Hel,WA	0.00000	(-0.00641,	0.01045)
25	24	0.01765	(0.00475,	0.03867)
24	27	0.00463	(0.00125,	0.01015)
27	Region7,ID	0.00000	(-0.00079,	0.00129)
27	26	0.00110	(0.00007,	0.00279)
26	Region6,ID	0.00033	(-0.00064,	0.00189)
26	Ranier,WA	0.00978	(0.00245,	0.02174)
24	Region4,ID	0.00000	(-0.00268,	0.00437)
23	S.Ukiah,OR	0.00000	(-0.00567,	0.00925)

Figure 15b. Branch lengths and confidence limits for the phylogenetic tree in Figure 15a. 741 trees examined, Ln Likelihood = 278.76

Chapter 7

DISCUSSION

> Not only does extensive variation take place among individual deer exposed to the same conditions of life,. but persistent differences in the condition of life become registered in the deer of a district by variation on some determinate line.
>
> A.G. Cameron, 1923
> The Wild Red Deer of Scotland

The results in this isozyme study of red deer and wapiti present a contrasting picture. On the one hand the polymorphic loci, six found in Scottish red deer and five in Northwest wapiti, indicated highly significant differences between locations. As the sampling area was expanded, the relative amount of variation which was due to differences between individuals dwindled, and that attributable to groups - locations, subspecies, etc. - steadily grew. When the final analysis incorporated both red deer and wapiti, the relative gene diversity of populations nearly equalled that of individuals. These results suggest not a homogenous gene pool, but many small populations, with subtle but distinct differences which are significant.

On the other hand, the absolute gene diversity, which is measured from the total random sample of electrophoretic loci, both polymorphic and monomorphic, remains small regardless from how far afield the samples come. (Recall one of the original reasons for comparing Scottish red deer and North American wapiti was that they represent not only morphological extremes but also the geographical extremes in

the present distribution of these animals.) The genetic distances which include all loci also show that red deer and wapiti are very similar, sharing at least 95% of the genome as sampled at this random selection of enzyme loci. With both red deer and wapiti, the general indices of electrophoretic variation are typical of mammals, but mammals have lower values than all other animal and plant groups (Nevo, 1978). Moreover, significant differences in average heterozygosity were found between red deer populations, and again between wapiti populations.

These have been perplexing results for one who began by simply watching wapiti (then called elk), strongly suspecting that there were differences between herds, and wanting to try and measure those differences systematically so that management might be more sensitive to local changes in populations. Enthusiasm at finding variation that could be counted and predicted, genotypes at polymorphic loci in Hardy-Weinberg equilibrium, was replaced with reflection on how this variation related to phenotypes, whole animals, and the behaviour of deer populations. The major points previously mentioned are discussed in turn, though not in sections as in earlier chapters, as the attempt here is to integrate them.

The evidence for genetic subdivision in red deer and wapiti came from three different though related sources. First, the observed genotype frequencies at most localities did not significantly deviate from Hardy-Weinberg proportions at any enzyme locus. In three years of sampling, there was only one locus in one population (SOD-1, Isle of Rhum, 1979) which did not meet Hardy- Weinberg predictions. When populations were combined, however, the two most variable loci (SOD-1 and IDH-2) were no longer in binomial proportions, in each case due to

a paucity of heterozygotes. Considering the strong evidence in other mammals that the alleles of these enzyme loci are inherited, the simplest explanation for the relative reduction in heterozygotes as the sampling area widened was that the deer sampled did not comprise a panmictic population, i.e. a common gene pool.

Secondly, the gene frequencies of polymorphic loci differed significantly across populations. Whether analysed using chi-square contingency tables or log-likelihood ratio tests, the frequencies for the same allele were significantly different at the majority of polymorphic loci. As these tests are additive, the cumulative results indicated highly significant heterogeneity between populations across all loci where variation was detected.

Thirdly, the relative measures of population subdivision suggested by Wright (1943) and Nei (1975) showed that an increasing amount of the variation observed was due to differences between populations as the animals sampled came from further afield. The lowest level of genetic differentiation recorded in Scottish red deer (F_{st} = 6.8%) is similar to that separating human races (F_{st} = 6.9%, Hartl, 1980, p.163). The relative genetic diversity increased to 22% when continental red deer were included in the analysis, and to 46% when wapiti and the same red deer were analysed together. Qualitatively, Hartl (1980, p.164) considered F_{st} values of 5-15% as moderate differentiation, and averages above 25% "very great differentiation." By this relative standard, the differentiation between red deer and wapiti is substantial.

Finding genetic subdivision in red deer and wapiti is not surprising. If inherited as well as environmental "conditions of life" are implied in the epigram which opens this chapter, Cameron had

essentially the same idea sixty years ago. Darling (1937, p.104-106) was more explicit in including social structure among the critical factors which have shaped red deer. With the advent of protein electrophoresis, McDougall and Lowe (1968) and Bergmann (1976) each found transferrin gene frequency differences in different red deer populations. Moreover, enzyme polymorphism - at some of the same loci which were polymorphic in this study - has been used to demonstrate genetic subdivision in other deer species in both North America and Europe (Manlove et al., 1976; Ryman et al., 1980).

Is the substantial genetic differentiation found between red deer and wapiti a sufficient argument for considering them to be separate species? It cannot be, for relative measures of population subdivision ignore the monomorphic loci (as they do not contribute to the between-population variance) and the polymorphic loci alone are not a random sample of the genome, one of the premises for using electrophoretic data to objectively compare different organisms (Lewontin, 1974). The absolute gene diversity, which takes all loci into account, is quite low for red deer and wapiti (Dm= 4.1%±2.3). In other words, although nearly half of the variation detected was due to differences between populations (or higher levels of organizations when the data was analysed hierarchically), all of the variation occurred in less than five percent of the genome sampled.

The reason for the low absolute gene diversity is apparent from a review of the gene frequency data. No fixed allele differences neatly divided the populations into two groups; SOD-1 came closest with the fast allele averaging 90% in wapiti and 12% in red deer. Only 7 of 28 loci sampled in all animals were polymorphic, and only 3 of these were polymorphic is both red deer and wapiti. Finally, only two alleles

were identified at each polymorphic locus, and the alternate allele was often rare with a frequency of less than 10% in most populations.

The genetic distance (Nei, 1972) separating red deer and wapiti is also low (D = 0.025), though four times the distance separating the most distinct Scottish red deer populations (D= 0.006) or those of Northwest wapiti (D = .005). Despite the small absolute distances, the pattern of branching in both dendrograms and phylogenetic trees was consistent with previous morphometric relationships. In addition to the division between red deer and wapiti (Lydekker, 1898; Cameron, 1923), the grouping of red deer from Scotland with those from Norway concurs with the findings of Ahlen (1965) and Gyllensten et al. (1982). The division of Roosevelt and Rocky Mountain wapiti has also long been recognized (Bailey, 1935; Murie, 1951). However, the confidence intervals for the branch lengths of these trees are large, and would be reduced in such closely related animals by substantially increasing the number of loci (Nei and Roychoudhury, 1974).

The genetic distance separating red deer and wapiti is less than that which divides European and North American moose (D= = 0.060, Reuterwall, 1980), which are considered conspecific. It is more than an order of magnitude less than the average genetic distance separating "operational taxonomic units" of other large grazing mammals (D = 0.849, Baccus et al., 1983, use this term to avoid the issue of what constitutes a species). Though about a third fewer loci were analysed in these cases, they were a random selection. By this comparative criterion then, red deer and wapiti should be considered a single species, Cervus elaphus L.

It should be added that the close relationship found between red deer and wapiti is not a result of an abnormally low level of

electrophoretic variation. At the outset of this study, the prevailing view was that large animals - particularly mammals - exhibited much lower levels of intraspecific genetic variation than other organisms, as measured in electrophoretic surveys. This view is supported by low heterozygosities recorded in several species: for example, two seal species (Mirounga angustirostris, M. leonina) (McDermid et al., 1972; Bonnel and Selander, 1974) at least two primate species (Pan troglodytes, Macaque fuscata) (King and Wilson, 1975; Nozawa et al., 1975) alligators (Alligator mississippiensis) (Gartside et al., 1977) and polar bears (Thalarctos maritinus) (Allendorf et al., 1979), The first enzyme studies on two deer species, moose (Ryman et al., 1977; Wilhelmson et al., 1978) and wapiti (Cameron and Vyse, 1978) were also in accord with this view.

Several theories have developed to explain the differences in the amount of isozyme variation in different animal groups. Selander and Kaufman (1973) applied Levins' (1968) theory of environmental amplitude: smaller, less mobile animals should experience habitats as alternatives and thus be more locally adapted. Sampling across habitats, different alleles would be favoured. In contrast, large mobile animals, which would encounter diverse habitats, would experience such differences as fine-grained, and thus be more generally adapted and have fewer allelic differences. Selection acting on enzyme variants is fundamental to their argument, as the authors state in concluding: "at least a major proportion of the allozymic variation in natural populations is maintained by natural selection." King and Wilson (1975) present another view, that variation at structural loci, such as enzyme and blood proteins , may be irrelevant to speciation. Finding that the genetic distance separating chimpanzees and humans is very small whether measured by

electrophoretic, immunological or amino acid sequencing techniques, they maintained that organismic and molecular evolution are independent, and that only a few substitutions in regulatory genes could account for large adaptive differences.

The variation found in this study of red deer and wapiti did not support the first contention that large mammals are more monomorphic at electrophoretic loci than either small mammals or other vertebrates. The general indices of genetic variation for Scottish red deer (H = 3.0%, P =0.137) and Northwest wapiti (H =2.5%, P = 0.124) fall close to average levels which Nevo (1978) and Baccus et al. (1983) found for mammals. These are conservative estimates, as the unweighted averages of all populations. If the red deer and wapiti sampled are treated as a single unit, the percentage of polymorphic loci (P = 0.178) is higher than the average found in mammals (P = 0.147, Nevo, 1978; P =0.128, Baccus et al., 1983), However, such lumping seemed unwarranted, favouring populations where more samples were collected. As analysis of enzyme proteins in culled animals has increased the number of large mammals for which these indices have been measured - particularly species that are not endangered - the environmental amplitude theory has been undermined (Ryman et al., 1980; Baccus et al., 1983). Furthermore, no evidence was found in Scottish red deer to sustain the foundation of the hypothesis, that selection is acting on electrophoretic loci.

The variation found in red deer and wapiti is consistent with the second hypothesis, that speciation events are decoupled from gene frequency differences at structural loci, and are instead due to changes in regulatory genes and/or chromosomal rearrangements (Goldschmidt, 1940; Britten and Davidson, 1969, 1971; Wilson et al.,

1977). Typical of mammals, red deer and wapiti do have lower heterozygosities than other animal classes. Speciation will be related to reproductive isolation, whether it is the cause or effect of genetic subdivision. Perhaps the slower, more regular clocks of structural gene loci have not caught up with the relatively recent proliferation of mammal species. Gould (1980), though not an originator of this view, states it succinctly: "The modern synthesis, as an exclusive proposition, has broken down on both of its fundamental claims: extrapolationism (gradual allelic substitution as a model for all evolutionary change) and nearly exclusive reliance on selection leading to adaptation. Evolution is a hierarchical process with complementary, but different, modes of change at its three major levels: variation within populations, speciation, and patterns of macroevolution. Speciation is not always an extension of gradual, adaptive allelic substitution to greater effect, but may represent as Goldschmidt argued, a different style of genetic change - rapid reorganization of the genome, perhaps non- adaptive."

Gould perhaps overstates the demise of the modern synthesis. It has been recognized for decades that evolutionary rates are not constant (Simpson, 1944) and that speciation can occur from small, isolated populations (Mayr, 1963), not only from the gradual transformation of large established lines. Stanley (1979) suggests that quantum speciation is not a new paradigm but a different emphasis in looking at the gaps in the fossil record, as well as gene frequency differences at structural loci in extant species.

It does provide one plausible explanation for the great difference between the relative and absolute gene diversity in red deer and wapiti. The isozyme variation detected reflects the breeding

patterns of these deer, but is only indirectly - and probably retroactively - related to morphological variation, adaptation or speciation. This view also makes intuitive sense to anyone who has looked at evidence of convergent evolution in a wide range of animals. In an example such as Bergmann's Rule (Mayr, 1963), a regulatory gene (or gene complex) for homeothermy suggests a more straightforward and hence widespread response to the colder climate than each Holarctic species body-building locus by locus.

If this is the case, then electrophoresis, at least of structural loci such as the enzymes analysed in this study, probably has far fewer taxonomic applications than its original promise suggested. It provides a no more absolute answer to whether wapiti or red deer are one species or two than the older minimal criterion of interbreeding (Mayr, 1963). And for those who follow the dictum of Medawar (1979) that "quantification has no merit as such except insofar as it helps solve problems," it raises the more general question of how applicable is the method to problems in deer biology.

Where the focus in wildlife management and animal behaviour is on intraspecific differences, i.e. those between individuals and/or populations, there are still several applications of electrophoresis. The conservation of both red deer and wapiti depends critically on the control of human predation - whether it be increasing culls in some areas or reducing them in others. The most obvious use of isozyme markers is in discriminating deer in the enforcement of game laws. This ought not be underestimated if the other uses are to eventually become commmon practice, as catching poachers is as high a priority for many game and estate managers as dispatching deer is for stalkers or hunters. It is a fortunate coincidence that the same freezer which

preserves game for the table preserves it for electrophoretic analysis as well. Once a deer had been skinned and boned it would, until recently, have been difficult to distinguish from other meat - except perhaps by tasting.

Frozen deer and beef muscle are readily differentiated by electrophoresis (Baccus et al., 1983), and similar differences have been found for blood proteins between deer and other domestic animals (Bunch et al., 1976). It is the monomorphic loci which are of particular importance in this type of forensic test, as it is only absolute differences rather than those of probability which would be beyond reasonable doubt and thus admissable as evidence (England: R. v. Murtagh and Kennedy, (1955); Scotland: McKenzie v. H.M. Advocate, 1959). There are fixed differences between red deer/wapiti and cattle at three of the loci in this study: G-6-PD, LDH-1, and 6-PGD. Polymorphic loci such as MDH-1 and PEP-2 which have no common alleles between these deer and domestic beef might also be used to make an expost facto case for poaching. There are also fixed differences between red and roe deer, as well as between wapiti and white-tail or black-tail deer (Baccus et al., 1983).

The second forensic application found for electrophoresis, though less pertinent to Scotland, is a tool that game managers in the United States have long sought to combat commercial poaching. Such poaching is sometimes done by taking one legal animal, and using the severed head of that animal to get several more illegally shot wapiti out of the woods and past the roadway checking stations which are used to monitor carcasses during the hunting season. Specifically, it is females which are usually shot and quartered so that the sex is obscured, and then brought out with the head of a young male. It is

the polymorphic loci which are useful in this case: if a muscle sample from the neck has a different genotype than one from the body, then there is strong evidence that the hunter has parts of two animals, though licensed only to shoot one. The only major assumption is that the isozymes are inherited.

But what is the likelihood of finding heads and tails that do differ electrophoretically, in different animals? This was calculated using the gene frequencies of wapiti on the Olympic Peninsula, where poaching is an acknowledged problem . The frequencies for SOD-1 at that locality were p = 0.383 and q = 0.617. Listed below are the corresponding genotype frequencies and the probability of two different wapiti from that area sharing the same genotype, simply the genotype frequency squared.

$$AA \times AA = (p^2 \times p^2) = (0.1467) \times (0.1467) = 0.0215$$

$$Aa \times Aa = (2pq \times 2pq) = (0.4726) \times (0.4726) = 0.2233$$

$$aa \times aa = (q^2 \times q^2) = (0.3807) \times (0.3807) = \underline{0.1449}$$

TOTAL 0.3898

The likelihood of two wapiti having the same genotype for SOD-1 is the sum of the three separate genotypes, or 39%, and the chance of them being different is thus 61%. As four polymorphic enzyme loci were detected on the Olympic Peninsula (SOD-1, MPI, PGM-1, PEP-2), the number of potential genotypes in any individual goes up dramatically ($3^4 = 81$), as does the probability of finding a genotypic difference in two wapiti. The calculations for each of the 81 genotypes are listed in Appendix 7. The resulting probability of finding a difference between two wapiti being passed off as one is 90%, so the chances of catching poachers who are using this technique is high indeed, and would only improve if other polymorphic proteins were

found.

The genetic variation found in red deer and wapiti has other, constructive uses beyond poacher detection. Significant differences in gene frequency could provide a rationale for management policies which closely reflect the biological units of red deer and wapiti. Genetic information in combination with data on population dynamics, which are also obtainable from culled samples, could make wildlife management more sensitive to local conditions. If carried out routinely for forensic purposes, electrophoresis could detect changes in population structure, as well as helping to answer fundamental questions in large mammal population genetics. For example, an estimated 2000 wapiti were killed by the 1980 eruption of Mt. St. Helens. Most biologists expected that the population on the north slope of the mountain would be decades rebuilding, considering the conservative home-range pattern of neighboring wapiti (Jenkins, 1980) and red deer. However, wapiti have already returned in numbers to St. Helens (Evelyn Merrill pers.com.; personal observation, July, 1983) and the game department has recently re-opened the hunting season there. It would be valuable to know where these animals came from and, ultimately, what made them change their usual ranging behaviour.

In addition to differences in gene frequency, significant differences were found in the amount of genetic variation between localities. Scottish red deer had significantly higher average heterozygosities than Continental populations (especially those from Norway where no variation was detected at 34 loci) and Northwest wapiti had higher heterozygosities than populations from the Rocky Mountains.

The most obvious genetic explanation for these differences is bottlenecks and/or founder effects - both mechanisms by which genetic variation is reduced during a constriction in population size. Nei (1975) and Motro and Thomson (1982) have shown theoretically that the increase in genetic variability will lag behind a return of animal abundance. Bottlenecks have been cited to explain low levels of electrophoretic variation in large mammals which have been hunted to near extinction, such as elephant seals (Bonnel and Selander, 1974) and cheetah (O'Brien et al., in prep.).

In highly manipulated deer species, it is tempting to attribute the low heterozygosity found in particular localities to human actions. The lack of variation in Pere David's deer (Ryder et al., 1981) which have been bred in captivity for centuries, and fallow deer introduced to Britain (Pemberton, 1983) have been explained by population reduction. In the case of Rocky Mountain wapiti, however, Cameron and Vyse (1978) maintained that the Yellowstone population has never numbered fewer than 4000 since Caucasion settlement. Acknowledging that overlapping generations, harem breeding and population subdivision could contribute to an effective population size substantially lower than the actual minimum number, they calculate that it still would have been several hundred animals.

Genetic bottlenecks and founder effects have not been systematically studied in large mammals, but it is testable in both red deer and wapiti. For example, the wapiti population of Afognak Island, Alaska was established with only eight individuals from the Olympic Peninsula, whereas the Michigan population was built up from 23 wapiti from Yellowstone. As mentioned in the previous chapter, the average heterozygosity for animals from the Olympic Peninsula is more

than twice that of the Yellowstone population (H = 3.9% and 1.2% respectively).

As with differences in gene frequency; differences in heterozygosity could also be considered in management policy. The case of the Mt. Ranier wapiti has arisen in the course of this study. The introduction of these animals from Yellowstone dates from an era when it was believed that more was better, especially when it meant more game animals (Gabrielson, 1941; Leopold, 1949). The National Park Service is now as concerned with protecting rare endemic plants as the wapiti, especially if they are not native. But hunting is prohibited in the National Parks, and these animals can be controlled only if it can be shown that they are exotic. Genetic markers could be useful in this regard. Though the sample size was small, the results of this study indicate that the Mt. Ranier animals not only have similar gene frequencies to their Yellowstone relatives, but they have the lower level of genetic variation. Since preserving genetic diversity is among the aims of the National Parks, the time may soon come to favour rare endemic plants over hungry, introduced wapiti which, though they may stray from Mt. Ranier, seem to find their way to the Park boundary when the shooting starts in November.

Electrophoresis might also be used in the management of captive deer, especially where breeding records are incomplete. Despite the claims that the deleterious effects of inbreeding have been exaggerated (Whitehead, 1980; Greig, 1979), researchers at the Smithsonian have compiled evidence of higher juvenile mortality in highly inbred ungulates (Ralls et al., 1979; Ralls et al., 1980; Ballou and Ralls, 1982). Fifteen of 16 ungulate species had higher juvenile mortality among highly inbred mating; Pere David's deer were

the exception that seems to prove the rule, with their long history in captivity (Wood-Jones, 1951-1952). In the absence of adequate records, as is often the case with captive deer, it would be prudent to breed deer with the highest heterozygosity, or pair individuals with the greatest gene frequency differences.

On the positive side, if there are heterotic effects in deer that can be correlated with valuable quantitative characters, then isozyme analysis might be utilized where deer now are reared intensively. There is only slight evidence of heterosis in this study - the effect of individual heterozygosity on antler point number - though the conditions in the Scottish Highlands may not be the place to test for the effects of heterosis. Smith et al. (in prep) also found a significant relationship between heterozygosity and alter point number in young white-tailed deer, though not in older age classes. The case for significant correlations between heterozygosity as measured by electrophoresis and quantitative characters such as antler points remains to be proved. There is, however, great potential for testing it in New Zealand, where red deer, wapiti and their hybrids are being bred in captivity.

The final utility of electrophoresis in wildlife management is perhaps not testable, but has been an impression which has grown stronger in the course of this study. Those responsible for the welfare of red deer and wapiti (stalkers, hunters, estate-owners, zoo-keepers, et al.) are much more readily persuaded by arguments based on data to which they have directly contributed (by providing blood samples or bits of tissue) than by biological theory, no matter how elegant. Whether electrophoresis will become a tool of conservation biology may depend ultimately on how well bands on gels

can be explained in terms of the behaviour of deer populations.

Chapter 8

CONCLUSION

In this study of isozyme variation in over 900 animals, polymorphism was detected in 6 of 34 enzyme loci in Scottish red deer and 5 of 28 loci in North American wapiti. Both the average heterozygosity (H) and the percentage of polymorphic loci (P) did not significantly differ from other large grazing mammals (Baccus et al., 1983) or mammals generally (Nevo, 1978).

The results do not support the contention that large mammals, being adapted to a wide range of habitats, have less isozyme variation than small mammals (Selander and Kaufman, 1973). Moreover, there was no strong evidence of selection at enzyme loci, a tenet of that hypothesis. The results are in agreement with previous findings (Nevo, 1978; Baccus et al., 1983) that mammals show less variation in structural loci than other animal classes.

Genetic subdivision characterizes the population structure of both red deer and wapiti. Chi-square and log-likelihood ratio tests showed highly significant heterogeneity between populations in both groups. There were also differences in average heterozygosity between populations: Scottish red deer showed significantly more variation than continental red deer, and native Northwest wapiti had significantly higher heterozygosities than introduced animals from the Rocky Mountains.

Measures of the standardized gene variation (F_{st}, Wright, 1965) showed moderate differentiation within Scottish red deer and Northwest

wapiti, and very great differentiation when the two groups were combined. Analysed hierarchically (Chakraborty et al., 1982) about half of the variation detected was due to differences between individuals (53.8%), a third due to differences between red deer and wapiti (32.2%) and the remainder was attributable to intermediate levels of organization (3.6% to populations and 10.4% to recognized subspecies).

Although a significant percentage of the electrophoretic variation detected is attributable to differences between red deer and wapiti, the absolute gene diversity - including monomorphic loci - confirms that these animals are closely related (Dm = 0.041) In other words, the red deer and wapiti sampled have more than 95% of their genome in common as sampled at 28 randomly selected loci.

The genetic distance (Nei, 1972) separating red deer and wapiti (D = 0.025) is similar to that between European and North American moose (D =0.060, Reuterwall, 1980), which are considered conspecific; it is more than an order of magnitude less than the average genetic distance between large grazing mammal species (D = 0.849, Baccus et al., 1983). These comparative results thus support considering red deer and wapiti one species, Cervus elaphus L. However, speciation may be caused by changes in regulatory genes or chromosomal rearrangements (Wilson et al., 1975) rather than the accumulating gene frequency differences at structural loci sampled in this study.

The electrophoretic variation detected has several potential applications to wildlife management, in differentiating both individuals and populations. In forensic use, deer can be distinguished from other meat by fixed allele differences at several monomorphic loci, and freezing of meat for later consumption also

preserves it for later laboratory analysis. The amount of variation typical of red deer and wapiti also makes it highly probable that two individuals can be discriminated from two parts of the same animal.

Significant gene frequency differences could be used to establish management units which more closely reflect the breeding pattern of subdivided deer populations, and significant differences in heterozygosity may distinguish native deer populations deserving protection from introduced animals requiring control. Lastly, electrophoretic markers may prove useful in developing breeding programs in captive deer populations, either where inbreeding depression has been documented (Ralls et al., 1979) or where wild deer are now being domesticated (Yerex, 1982).

Conservation genetics is still a young discipline (Frankel and Soule, 1981) and electrophoresis has only begun to be applied to management problems in game animals such as deer (Manlove, 1976). Although electrophoresis may have been over-rated as a method of measuring large scale evolutionary change, it has been under-utilized as a tool in managing wild populations. Whether this potential will be realized for red deer and wapiti populations depends largely on the public will to preserve not deer numbers, but deer diversity.

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APPENDIX 1

In this systematic summary of research in cervid biochemical genetics, the papers are organized first according to taxonomic family and secondly according to recognized species or semispecies.

FAMILY CERVINAE

Cervus elaphus Red deer

Red deer are among the most extensively studied of deer species and electrophoretic variation has been found in both blood and enzymatic proteins. Transferrin polymorphism has been found in most red deer populations where it has been studied. Lowe and McDougall (1961) identified two transferrin alleles in Scottish red deer and subsequently demonstrated gene frequency differences between two Scottish populations (McDougall and Lowe, 1968). Bergmann (1976) found three transferrin alleles, and significant differences between German red deer populations, though several localities were not in Hardy-Weinberg equilibrium.

Analysing 138 Russian red deer, Kravchenko and Kravchenko (1971) reported four transferrin alleles in 138 Russian red deer, as well as proposing possible variation in three other serum protein systems. Wegge (1978) found two transferrin alleles on an island population in Norway, though a nearby mainland locality was monomorphic. Gyllensten et al. (1980) developed an agarose technique which discriminated three transferrin alleles in red deer from Sweden, and the authors demonstrated significant spatial heterogeneity between populations.

Enzymatic loci analysed from tissue samples of culled red deer have shown considerable variation. Gyllensten et al (1982), comparing populations from four supposed subspecies, found six polymorphic enzymes: GPI-1, IDH-2, MDH-1, PGM-1, PGM-2 and SOD-1. Baccus et al. (1983) confirmed the IDH and MDH polymorphism and Holt (1977) found variation at IDH-2, PGM-2, as well as MPI. Polymorphism has also been found for a whey protein in milk from Scottish red deer (McDougall and Stewart, 1976).

Wapiti

In contrast to red deer, there has been less evidence of variation in blood proteins of North American wapiti. Although transferrin polymorphism was reported by Miller et al. (1965), subsequent studies (Johnson, 1968; Cameron and Vyse, 1978) have not substantiated it from other samples. Both albumin (Cameron and Vyse, 1978; McClymont et al., 1982) and haemoglobin (Butcher and Hawkey, 1977; Bunch et al., 1981) have also thus far proved monomorphic. Successive enzyme surveys, however, have each found additional polymorphic loci. Cameron and Vyse (1978) found variation only at IDH-2. Baccus et al. (1983) detected polymorphism at both IDH-2 and MPI, sampling at a different locality. Dratch and Gyllensten (in press), testing eleven populations, found variation at PEP-2, PGM-1 and SOD-1 as well as the two previously described polymorphic loci.

Sika deer

Kravchenko et al. (1971) identified three transferrin alleles in yy deer from Russia.. Lowe and McDougall (1961) found no transferrin polymorphism in the five yy they tested initially, nor in ten sampled later (McDougall and Lowe, 1968). Electrophoresis of 44 yy deer

showed no haemoglobin variation (Maughan and Williams, 1967), but two isoelectric focusing studies on fewer samples (Butcher and Hawkey, 1977; Lawton and Sutton, 1981) have found polymorphism. Of seventeen enzymatic loci surveyed, Holt (1977) found variation at IDH-2, MPI, PGM-2 and PGD. Three of these enzyme loci have shown polymorphism in red deer and/or wapiti.

Elaphurus davidianus Pere David's deer

No polymorphism has yet been found in this relic species. Haemoglobin has been studied in only ten individuals by isoelectric focusing (Butcher and Hawkey, 1977; Lawton and Sutton, 1981), and an electrophoretic analysis of plasma proteins sampled twenty Pere David's deer (Ryder et al., 1981).

Dama dama Fallow deer

Despite widespread coat colour variation, fallow deer are notable for their lack of electrophoretic variation. Early work revealed no transferrin polymorphism in 77 fallow from British deer parks (Lowe and McDougall, 1961; McDougall and Lowe, 1968). A recent survey of 367 fallow confirmed this finding for transferrin as well as other serum proteins (Pemberton, 1983). No haemoglobin polymorphism was found by gel electrophoresis of 62 samples (Maughan and Williams, 1962) or isoelectric focusing of 26 samples (Lawton and Sutton, 1981). Among enzyme loci, Munday (1974) found no variation at the LDH locus in fifteen fallow deer, and Holt (1977), analysing 17 enzymatic loci, found the only possible variation at AK- 2. Screening at least 100 at 26 loci (including AK-2) Pemberton (1983) again found no variation.

Other Cervinae species

Only haemoglobin has been analysed in four other Cervinae species. Listed above are the number of banding patterns observed for each species, followed parenthetically by the number of individuals tested. Blood samples were taken from captive animals primarily for intraspecific comparison. Thus sample sizes are low, and can at best suggest haemoglobin variation in several cases (particularly for Axis axis). The first two studies in the table employed conventional electrophoresis, the latter two used isoelectric focusing.

Axis	Barasinga	Timor	Hog	Reference
2(4)	-	-	-	Naik et al., 1964
1(1	1(1)	-	-	Maughan and Williams 1967
2(3)	1(7)	2(3)	1(1)	Butcher and Hawkey, 1977
1(5)	2(5)	-	1(1)	Lawton and Sutton, 1981

FAMILY ODOCOILEINAE

Rangifer tarandus Reindeer

More alleles have been found for reindeer transferrin than at any other deer locus. They are summerized chronologically in the table below. The number of populations tested is in parentheses following the number of individuals sampled.

Sample size	Location	Trf alleles	Reference
64 (1)	Sweden	3	Gahne Rendel, 1961
132 (2)	Norway	6	Braend, 1964a
829 (5)	"	8	Braend, 1964b
182 (1)	N.W. Russia	5	Shubin, 1969
408 (2)	"	5	Shubin Turubanov, 1970
502 (1)	"	7	Turubanov Shubin, 1971
1756 (16)	Siberia	9	Zhurkevich Fomicheva, 1976
81 (1)	Spitzbergen	2	Storset et al., 1978

In the only case where alleles were compared between studies, the two alleles found in Spitzbergen reindeer by Storset et al. (1978) were both of different mobility to all eight alleles found by

Braend (1964b). Using the large number of transferrin alleles found in Norway and Siberia, Braend (1964b) and Zhurkevich and Fomicheva (1976) demonstrated genetic divergence between herds. The latter authors also reported evidence for selection acting on transferrin phenotypes.

No polymorphism has yet been observed in serum proteins other than transferrin (Gahne Rendel, 1961; Storset et al., 1978); nor was haemoglobin variable in the few zoo reindeer examined by Butcher and Hawkey (1977) and Lawton Sutton (1981). Storset et al. (1978) found no polymorphism among seven enzyme loci screened in Spitzbergen reindeer, but Baccus et al. (1983) recently reported one polymorphic locus, MPI, of 19 screened in Swedish reindeer.

Caribou

Nadler et al. (1967), collecting sera samples from 37 caribou, found transferrin polymorphism suggesting three alleles; polymorphism for a second serum protein on the same gels was reported but not identified. Baccus et al. (1983) found no variation in four Alaskan caribou over 19 loci tested, but did report a different fixed albumin allele when comparing these caribou with the Swedish reindeer mentioned previously.

Alces alces European moose

Initial studies of European moose from Scandinavia (Braend, 1962; Wilhelmson et al., 1978) and from Russia (Shubin, 1969) failed to reveal any serum protein polymorphism, but more recently Gyllensten et al (1980), using agarose gels, detected a rare slow transferrin allele in moose from six out of 16 Scandinavian sampling sites.

Screening enzymes in red blood cells, a low level of genetic variation in the European moose (Wilhelmson et al., 1978 ; Ryman et al., 1977) was again indicated. Later, more extensive surveys using tissue samples, however, described allelic variation at MDH- 2, PEP-B, PGI-1, PGM-2 and MPI in Scandinavian animals (Ryman et al., 1980; Baccus et al., 1983). Gene frequency difference at these loci have been used to examine genetic divergence of moose populations between sample sites (Ryman et al., 1980) and between hunt compartments within sample sites (Chesser et al., 1982).

North American moose

Several studies of North American moose blood proteins (Nadler et al., 1967; Seal and Karns, reported in LeResche et al., 1974; Wilhelmson et al., 1978; McClymont et al., 1982) have failed to find any variation. Two of the three enzyme studies (Wilhelmson et al., 1978; Dilworth and Mackenzie, 1970) have shown the same negative result. Reuterwall (1980), however, analysed 47 samples from a Canadian population and found four polymorphic loci (LDH-1, MDH-2, PGD and MPI) among 15 loci screened.

Odocoileus virginianus White-tailed deer

There are more studies reporting electrophoretic research on white-tailed deer than on any other species. The pioneering work on Odocoileus serum protein variation was conducted by Cowan and Johnston (1962) and Van Tets and Cowan (1966), but since the protein bands were not identified, indirect comparison with later work is not possible. In subsequent studies, listed below, both transferrin and haemoglobin have generally proved polymorphic.

Haemoglobin has been the subject of intensive investigation in white-tailed deer, as sickling is widespread (Wilhelmson, 1964), and research has been aimed at relating it to particular haemoglobin variants. Two types of haemoglobin polymorphism have been reported: allelic variation at the Beta-haemoglobin locus and non-allelic variation in alpha chain expression. The number of transferrin and beta-haemoglobin alleles detected in different studies is tabulated in the table below; where alpha-chain expression has been noted, an "a" follows the number of Beta-haemoglbin alleles.

Sample size (sites)	Location	Alleles Trf	Alleles Hb	Reference
647+ (2)	Florida	-	4 a	Kitchen et al.., 1964
200 (4)	Iowa	2	4	Miller et al., 1965
700+ (2)	Florida	-	6 a	Kitchen et al., 1966, 1967
186 (7)	S.E. USA	-	6 a	Huisman et al., 1968
146 (1)	Minnesota	1	2	Seal and Erickson, 1969
88 (1)	Iowa	7	-	Quinteros and Miller, 1969
30 (1)	Florida	-	1 a	Taylor et al., 1972
421 (24)	S.E. USA	-	5 a	Harris et al., 1973
40 (1)	Michigan	2	-	LeResche et al., 1974
400 (1)	S. Carolina	3	4 a	Manlove et al., 1975
218 (2)	"	3	4	Ramsay et al., 1979
2455 (5)	"	-	4	Chesser et al., 1982
1000+ (8)	S.E. USA	3	4	Smith et al., (in press)

In contrast, albumin variants are rare or absent in white- tailed deer (Seal and Erickson, 1969; Manlove et al., 1976; McClymont et al., 1982; Smith et al., in press), though heterozygote band patterns were obtained in putative white-tailed deer x mule deer hybrids (McClymont et al., 1982). One other serum protein polymorphism has been noted, for an unnamed foetal protein (Seal and Erickson, 1969).

Beginning about a decade after the research started on blood proteins, several surveys of enzyme loci have also shown abundant variation in white-tailed deer (Manlove et al., 1975; Price et al., 1979; Baccus et al., 1983). Much of the work on these deer has

recently been summerized by Smith et al. (in press) reporting variation in 27 of 35 loci analysed, a much higher percentage than in any other deer species. These higher rates of polymorphism, in both blood and enzyme proteins, have stimulated research on ecological and reproductive correlates with differences in gene frequency and average heterozygosity.

Ramsey et al. (1979) showed that deer herds in swamp and upland habitats differed both genetically and demographically. Baccus et al. (1979) analysed data for the four allele SDH locus in seven populations and found evidence for selection at certain stages of the life cycle. Considering selection from an individual viewpoint, Johns et al. (1977) were able to determine that females carrying twin foetusses had significantly higher average heterozygosities than those carrying a single foetus. Smith et al. (in press), in a similar analysis of 640 males, found that more heterozygous animals had significantly greater body weights, kidney fat indices, and more antler points.

Odocoileus hemionus Black-tail and mule deer

As with white-tails, the early studies of Cowan and Johnston (1962) and Van Tets and Cowan (1966) suggest variation but did not identify the proteins involved. In more recent studies, Bunch et al. (1976) found evidence of two haemoglobin alleles in 36 samples, while McClymont et al. (1982) found no albumin variation in 100 mule deer. Baccus et al. (1983) screening two deer at 19 enzyme loci, claim polymorphism for MDH-2, and 6PGD.

Capriolis capriolis Roe deer

Though they have an extenive northern range, there have been very few genetic studies of these small deer. Gyllensten et al. (1980) found no transferrin polymorphism in 33 Swedish roe deer. In British animals, no haemoglobin variation was detected by electrophoresis of 80 deer (Maughan and Williams, 1967) or by isoelectric focusing seven other samples (Lawton and Sutton, 1981). The only electrophoretic variant described in roe deer to date is an enzyme polymorphism at locus PGM-1 (Holt, 1977)

Ozotoceros bezarctus Pampas deer

In a sample of 36 pampas deer, Quinteros et al. (1971) found seventeen transferrin phenotypes; the number of alleles responsible was not indicated.

FAMILY HYDROPOTINAE

Hydropotes inermis Chinese water deer

Maughan and Williams (1967) and Maughan (1969) report the only research sampling more than one Hydropotes. A slow haemoglobin variant was found in three of 21 feral animals sampled in Britain.

FAMILY MUNTIACINAE

Muntiacus reevesii Reeve's muntjac

In the only work sampling more than two muntjac, Maughan and Williams (1967) and Maughan (1969) described haemoglobin polymorphism in 40 feral Reeve's muntjac, with two alleles showing almost equal gene freqencies. Butcher and Hawkey (1977) claim a haemoglobin

polymorphism in M. reevsii on the basis of one animal, though they were comparing with several other deer species.

The table on the following page summarises the polymorphism detected at specific enzyme loci in the six species where extensive surveys have been undertaken. White-tailed deer have the highest percentage of polymorphic loci (p) and average number of alleles (A) per locus. Fallow deer also of intermediate size have the lowest values for both of these general indices of genetic variation.

LOCUS	SPECIES						%
	Cervus elaphus	Dama dama	Alces alces	Rangifer terandus	Capriolus capriolus	Odocoileus virginianus	
AP	1	1	1	1	-	2	20
ACON	-	-	1	-	-	-	
ADA-1	1	-	1	-	-	-	
ADA-2	1	-	-	-	-	-	
ADH	-	-	-	-	-	2	
AK-1	1	1	1	-	1	-	0
AK-2	1	2	-	-	1	2	50
CA-1	-	1	1	-	-	-	
CA-2	-	1	-	-	-	-	
CK	1	-	-	-	-	-	
DIA	-	-	1	-	-	-	
EST-1	3	1	1	1	1	2	33
EST-2	1	1	1	-	-	1	0
EST-3	1	-	1	-	-	4	33
EST-4	-	-	-	-	-	1	
EST-D	-	-	1	1	-	-	
GAPDH	1	-	-	-	-	-	
GDH	-	1	-	1	-	-	
GLO	-	-	-	1	-	-	
GLUT	1	-	1	-	-	2	33
GOT-1	1	1	1	1	1	2	16
GOT-2	1	1	1	1	1	3	16
GPD-1	1	1	-	-	-	3	33
GPD-2	1	1	-	-	-	3	33
G6PDH	1	1	1	1	1	2	16
GPI-1	2	1	2	1	1	1	33
GPI-2	1	1	1	-	-	1	0
GPT	1	-	1	1	-	-	0
GUS	1	-	-	-	-	-	
HK-1	1	-	-	-	-	-	
HK-2	1	-	-	-	-	-	
IDH-1	1	1	1	-	1	2	25
IDH-2	2	1	1	2	1	3	50
LDH-1	1	1	1	1	1	2	16
LDH-2	1	1	2	1	1	3	33
MDH-1	1	1	1	1	1	1	16
MDH-2	4	1	2	1	1	3	50
ME-1	2	-	1	1	1	2	40
ME-2	2	-	2	1	1	3	60
MPI	3	1	4	2	2	3	84
PEP-A	-	-	2	-	-	-	
PEP-B	2	-	1	1	2	2	60
PEP-C	-	-	1	-	-	-	
PEP-D	-	-	1	-	-	-	
6PGD	2	1	3	1	1	2	50
PGM-1	2	1	2	1	2	4	60
PGM-2	2	1	1	-	1	3	40
PGM-3	-	-	1	-	-	-	
PK	1	-	-	-	-	-	
SDH	1	1	1	1	1	4	16
SOD-1	2	1	1	1	1	4	33
SOD-2	1	1	-	-	1	-	0

Number of alleles per locus in the six cervid species in which isozyme surveys have been conducted.

APPENDIX 2

BUFFER RECIPES

1. ACID CITRATE (Clayton and Tretiak, 1972)

Electrode:	Citric acid	5.4g
pH 6.1	Distilled water	1.0l
	N-(3-Amino propyl)morpyline	10ml
Gel:	Acid citrate electrode buffer	200ml
pH 6.0	Distilled water	3800ml
pH adjusted with N-(3-Amino propyl) morphyline		

2. CONTINUOUS TRIS CITRATE (Selander et al., 1969)

Electrode:	Tris	83.2g
pH 8.0	Citric acid	30.0g
	Distilled water	1.0l
pH adjusted with 1.0M Sodium hydroxide		
Gel:	Tris Citrate electrode buffer	200ml
pH 8.0	Distilled water	5800ml

3. DISCONTINUOUS TRIS CITRATE (Selander et al,. 1969)

Electrode:	Boric acid	18.55g
pH 8.2	Sodium hydroxide	2.40g
	Distilled water	1.001
pH adjusted with 1.0M Sodium hydroxide		
Gel:	Tris	9.21g
pH 8.7	Citric acid	1.05g
	Distilled water	1.001

4. TRIS MALEATE (Selander et al,. 1969)

Electrode:	Tris	12.10g
pH 7.4	Maleic acid	11.60g
	Ethylene-diamine tetraacetic acid	3.72g
	Magnesium cloride	2.03g
	Distilled water	1.001
Gel:	Tris malaeic electrode buffer	4001
pH 7.4	Distilled water	36001

5. LITHIUM HYDROXIDE (Ridgeway et al., 1970)

Electrode:	Boric acid	18.54g
pH 8.5	Lithium hydroxide	2.52g
	Distilled water	800-10001
Gel:	Tris	36.25g
pH 8.0	Citric acid	10.50g
	Lithium hydroxide electrode	1001
	Distilled water	8001
	Dilute 1:20 with Distilled water	

ENZYME LOCI ANALYSED

ENZYME	ABBREVIATION	LOCI/ ALLELES	BUFFER	TISSUE
Acid phosphatase	AP EC 3.1.3.1	1/1	3	M
Adenosine deaminase	ADA EC 3.5.4.4	2/1,1	1	M K
Adenylate kinase	AK EC 2.7.7.3	2/1,1	1	M
Creatine kinase	CK EC 2.7.3.2	1/1	5	M
Esterase	EST	3/1,1,1	5	K
Glucose phosphate isomerase	GPI EC 5.3.1.9	2/2,1	5	K
Beta-glucuronidase	GUS EC 5.3.1.31	1/1	1	K
Glutamate dehydrogenase	GDH EC 1.4.1.2	1/1	1	K
Glutamate pyruvate transaminase	GPT EC 2.6.1.2	1/1	5	M
Glyceraldehyde phosphate dehydrogenase	GAPDH EC 1.2.1.12	1/1	5	M
Glycerol-3-phosphate dehydrogenase	GPD EC 1.1.1.8	1/1	5	M
Hexokinase	HK EC 2.7.1.1	2/1,1	3	M K
Isocitrate dehydrogenase	IDH EC 1.1.1.42	2/1,2	1,2	M K
Lactate dehydrogenase	LDH EC 1.1.1.27	2/1,1	5	M
Malate dehydrogenase	MDH EC 1.1.1.37	2/2,1	1	M
Malic enzyme	ME EC 1.1.1.40	1/2	5	M
Mannose phosphate isomerase	MPI EC 5.3.1.8	1/2	1	M K
Peptidase Leu-gly-glycine	PEP-2 EC	1/2	3	M
Phosphoglucomutase	PGM EC 2.7.5.1	2/2,2	1,4	M K
Phosphogluconate dehydrogenase	PGDH EC 1.1.1.44	1/1	1	M K
Pyruvate kinase	PK EC 2.7.1.40	1/1	1	M K
Sorbitol dehydrogenase	SDH EC 1.1.1.14	1/1	1	M K
Superoxide dismutase	SOD EC 1.15.1.1	2/2,1	1	M K

APPENDIX 4a

Heterogeneity Tests

An example of the BMDP program, used to test population heterogeneity from allele frequencies. Values are pN and qN.

```
BMDP4F
TWO-WAY FREQUENCY TABLES -- MEASURES OF ASSOCIATION
MULTIWAY FREQUENCY TABLES -- LOGLINEAR MODELS (INCLUDING STRUCTURAL
ZEROS)

DEPARTMENT OF BIOMATHEMATICS
UNIVERSITY OF CALIFORNIA, LOS ANGELES, CA 90024
PROGRAM REVISED JUNE 1981
MANUAL REVISED -- 1981
PLU VERSION 5.0, FEBRUARY 1982
COPYRIGHT (C) 1981 REGENTS OF UNIVERSITY OF CALIFORNIA
   AUGUST  8, 1983  AT 19:38:40

PROGRAM CONTROL INFORMATION

/PROBLEM  TITLE IS 'CHISQ / G TESTS'.
/INPUT  VARIABLES ARE 2.
        TABLE IS 2,6.
        FORMAT IS FREE.
        UNIT IS 10.

/VARIABLE NAMES ARE GENES,POPS.
/TABLE    COLUMN IS GENES.
         ROW IS POPS.
/CATEGORY CODES(1) ARE 1,2.
        NAMES(1) ARE P,Q.
        CODES(2) ARE 1 TO 6.
        NAMES(2) ARE CAITHNESS,ROSS,LAGGAN,STRATHM,RHUM,PERTHS.
/STATISTICS  CHISQ.
        FISH.
        LRCHI.
/PRINT EXP.
       DIFF.
       CHISQ.
       LRCHI.
/END.
```

***** OBSERVED FREQUENCY TABLE 1

POPS	GENES		
	P	Q	TOTAL
CAITHNES	19	21	40
ROSS	11	51	62
LAGGAN	5	33	38
STRATHM	11	15	26
RHUM	37	45	82
PERTHS	16	40	56
TOTAL	99	205	304

MINIMUM ESTIMATED EXPECTED VALUE IS 8.47

STATISTIC	VALUE	D.F.	PROB.
PEARSON CHISQUARE	24.201	5	0.0002

STATISTIC	VALUE	D.F.	PROB.
LIKELIHOOD-RATIO CHISQ.	25.449	5	0.0001

***** EXPECTED VALUES -- TABLE 1

POPS	GENES		
	P	Q	TOTAL
CAITHNES	13.0	27.0	40.0
ROSS	20.2	41.8	62.0
LAGGAN	12.4	25.6	38.0
STRATHM	8.5	17.5	26.0
RHUM	26.7	55.3	82.0
PERTHS	18.2	37.8	56.0
TOTAL	99.0	205.0	304.0

***** DIFFERENCES = OBSERVED - EXPECTED -- TABLE 1

POPS	GENES		
	P	Q	TOTAL
CAITHNES	6.0	-6.0	0.0
ROSS	-9.2	9.2	0.0
LAGGAN	-7.4	7.4	0.0
STRATHM	2.5	-2.5	0.0
RHUM	10.3	-10.3	0.0
PERTHS	-2.2	2.2	0.0
TOTAL	0.0	0.0	0.0

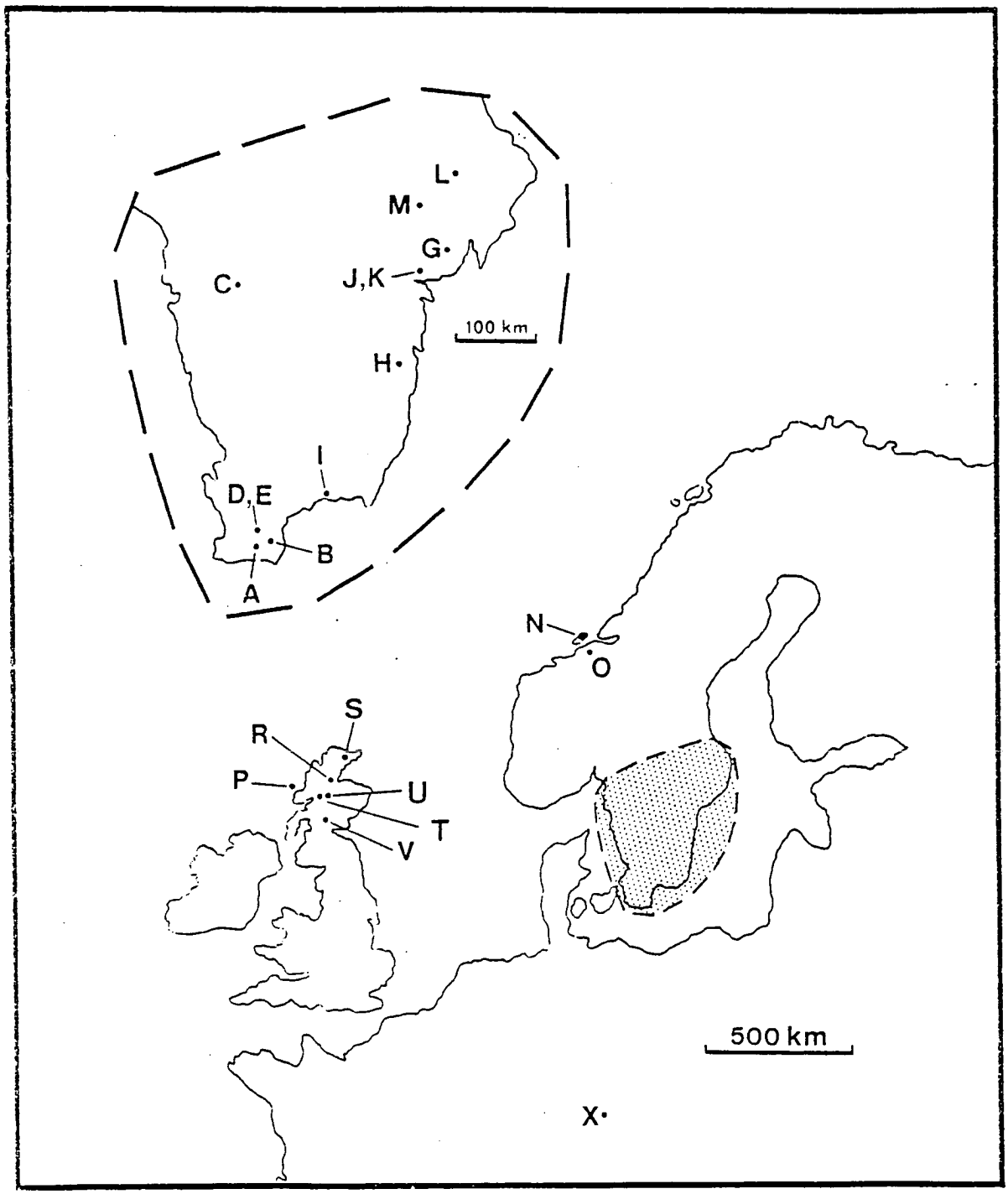
***** COMPONENTS OF CHI SQUARE = (OBS - EXP)**2 /EXP -- TABLE 1

POPS	GENES P	Q	TOTAL
CAITHNES	2.7	1.3 I	4.1
ROSS	4.2	2.0 I	6.2
LAGGAN	4.4	2.1 I	6.5
STRATHM	0.8	0.4 I	1.1
RHUM	4.0	1.9 I	5.9
PERTHS	0.3	0.1 I	0.4
TOTAL	16.3	7.9 I	24.2

** COMPONENTS OF LIKELIHOOD-RATIO CHI SQUARE = -2.0*OBS*LN(OBS/EXP)

POPS	GENES P	Q	TOTAL
CAITHNES	-14.3	10.5 I	-3.8
ROSS	13.4	-20.3 I	-6.9
LAGGAN	9.1	-16.7 I	-7.6
STRATHM	-5.8	4.7 I	-1.1
RHUM	-24.1	18.5 I	-5.6
PERTHS	4.2	-4.6 I	-0.4
TOTAL	-17.6	-7.8 I	-25.4

APPENDIX 4b.

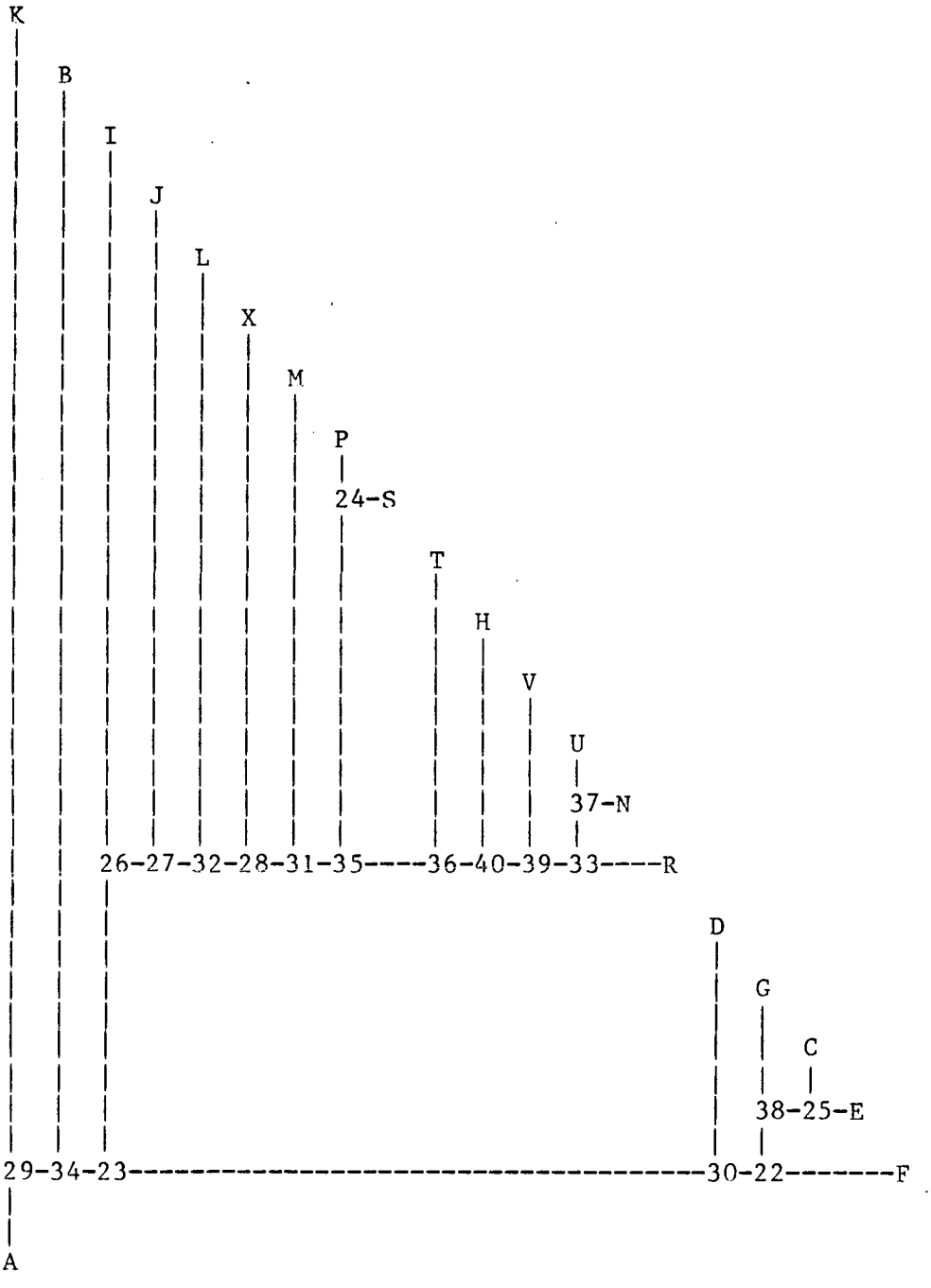


APPENDIX 4b. Location of red deer collection sites for comparison of of northern Europe subspecies, 1980. Names of sites and countries on the following page.

Map code	Collection site	Country	Subspecies
A	Red Deer Reserve (Vomb, Bellinga, Krageholm, Sövde)	Sweden	*C.e. elaphus*
B	Christinehof	"	"
C	Hunneberg	"	"
D	Skåne Deer Park	"	"
E	Skåne Deer Park (central enclosure)	"	"
F	Skåne, unspecified	"	"
G	Öster-Malma	"	"
H	Ankarsrum	"	Possibly mixed origin (*C.e. elaphus* and possibl other north European sub-species)
I	Eriksberg (enclosure)	"	"
J	Kolmården (free ranging)	"	"
K	" (enclosure)	"	"
L	Enköping	"	"
M	Västerås	"	"
N	Hitra (island population)	Norway	*C.e. atlanticus*
O	Sør Trøndelag	"	"
P	Rhum (island population)	Great Britain	*C.e. scoticus*
R	Ross-shire	"	"
S	Caithness	"	"
T	Loch Loggan	"	"
U	Strathmashie	"	"
V	Perthshire	"	"
X	Nationalpark Bayerische Wald	West Germany	*C.e. germanicus*

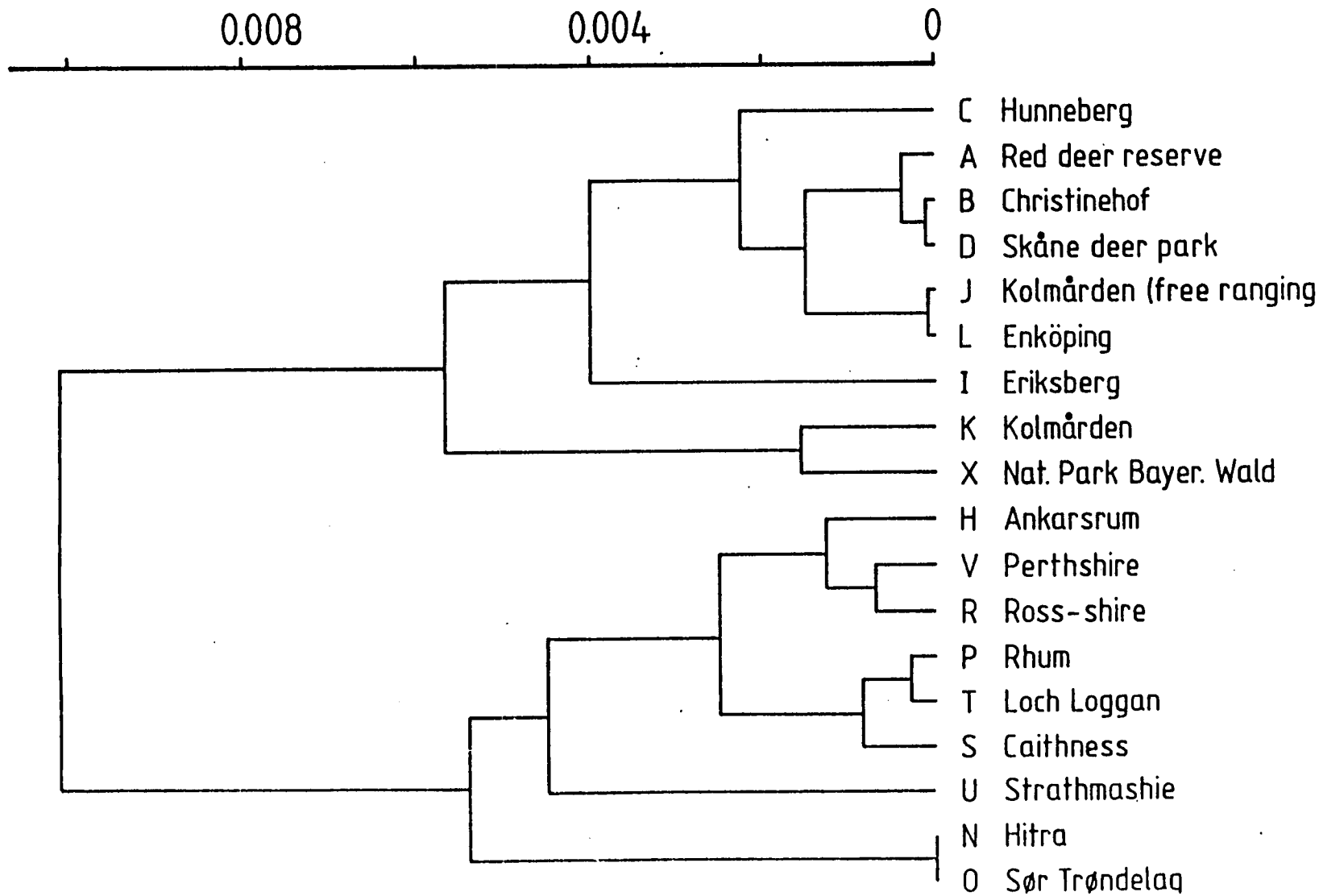
APPENDIX 4b. Collections sites for comparison of northern European red deer.

APPENDIX 4c.



Remember, this is an unrooted tree!
Ln Likelihood = 420.02307
Examined 721 trees

Appendix 4c. Comparison of genetic distance dendrogram (Nei, 1972) with phylogenetic tree using CONTML (Felsenstein, 1981) for northern European red deer.



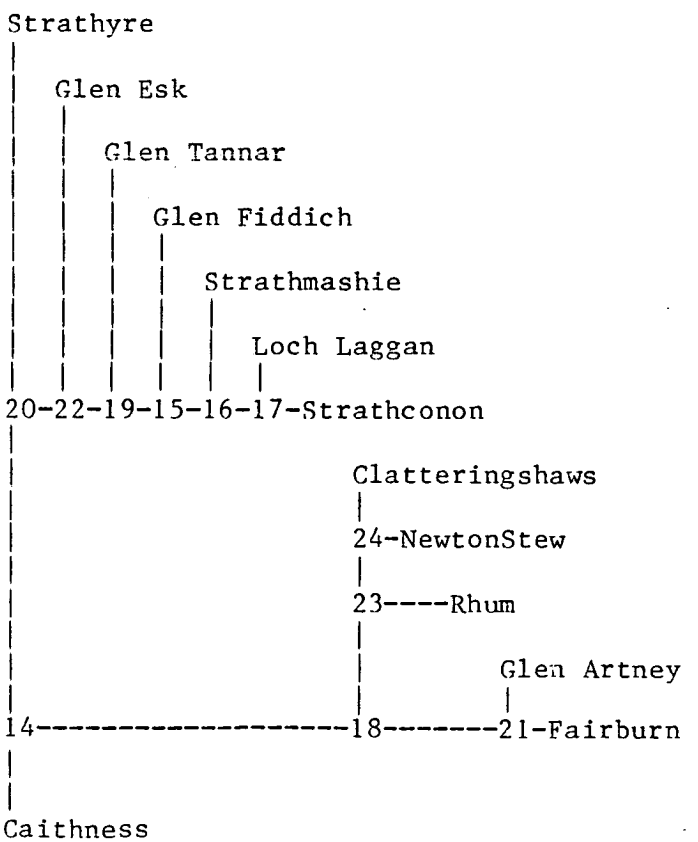
APPENDIX 4c. Dendrogram of genetic distance (Nei, 1972) of northern European red deer. Letters denote collection sites marked in the previous appendix and following tree.

APPENDIX 4d

Continuous character Maximum Likelihood method version 2.3
Felsenstein (1981). Phenotypes are arcsine square-root
transformations of gene frequencies.

13 Populations, 3 Phenotypes

Caithness	0.63100	1.44000	1.23920
Strathcon	0.32180	1.45220	0.95040
Fairburn	0.44330	1.28590	1.57080
GlenFiddic	0.52360	1.43250	0.98250
Strathmash	0.53160	1.57080	0.95990
LochLaggan	0.45360	1.57080	0.98250
Rhum	0.75940	1.22480	1.45650
GlenTannar	0.56090	1.31300	0.98680
Strathyre	0.63510	1.41530	1.09350
GlenArtney	0.57960	1.34530	1.57080
Glen Esk	0.65910	1.42540	0.93360
NewtonStew	0.89430	1.30700	1.57080
Clatrngshw	0.94400	1.12230	1.57080



Remember, this is an unrooted tree!

Branch lengths and confidence limits.

Ln Likelihood = 67.54187

Examined 231 trees

Between	And	Length	Approx. Confidence Limits
Caithness	14	0.00000	(-0.00562, 0.01261)
14	20	0.00729	(0.00044, 0.02264)
20	Strathyre	0.00000	(-0.00321, 0.00721)
20	22	0.00495	(-0.00110, 0.01853)
22	Glen Esk	0.00380	(-0.00131, 0.01526)
22	19	0.00121	(-0.00312, 0.01091)
19	GlenTannar	0.00319	(-0.00100, 0.01259)
19	15	0.00204	(-0.00141, 0.00979)
15	GlenFiddic	0.00000	(-0.00221, 0.00495)
15	16	0.00619	(0.00008, 0.01990)
16	Strathmash	0.00038	(-0.00130, 0.00414)
16	17	0.00182	(-0.00022, 0.00641)
17	LochLaggan	0.00000	(-0.00170, 0.00382)
17	Strathcon	0.01082	(0.00065, 0.03364)
14	18	0.02861	(-0.00279, 0.09906)
18	23	0.00806	(-0.00741, 0.04278)
23	24	0.00985	(-0.00144, 0.03518)
24	Clatrngshw	0.00937	(-0.00150, 0.03375)
24	NewtonStew	0.00283	(-0.00434, 0.01890)
23	Rhum	0.00000	(-0.00653, 0.01465)
18	21	0.01191	(-0.00519, 0.05028)
21	GlenArtney	0.00000	(-0.00493, 0.01106)
21	Fairburn	0.00737	(0.00045, 0.02290)

APPENDIX 4d. Branch lengths and confidence intervals for tree from the previous page. Data from thirteen localities sampled in Scotland in 1980.

APPENDIX 5

LSML76 - MIXED MODEL LEAST SQUARES PROGRAM
ABRO VERSION - 14 OCTOBER 1980
ADAPTED FROM HARVEY (1960)

OVERALL MEANS AND STANDARD DEVIATIONS OF RHM

WEIGHT	MEAN=	161.74129	S.D.=	46.10751
POINTS	MEAN=	4.90050	S.D.=	2.86706

COMBINED LEAST-SQUARES ANALYSIS OF VARIANCE

WEIGHT

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F	PROB
LOCATS	13	52096.02	4007.38	7.916	0.0000
HET %	2	342.08	171.04	0.338	0.7137
REGRESSIONS					
AGE B LINEAR	1	67071.04	67071.04	132.482	0.0000
AGE B QUAD	1	25285.20	25285.20	49.945	0.0000

POINTS

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F	PROB
LOCATS	13	150.34	11.56	1.755	0.0534
HET %	2	44.07	22.03	3.344	0.0375
REGRESSIONS					
AGE B LINEAR	1	5.22	5.22	0.793	0.3745
AGE B QUAD	1	45.85	45.85	6.957	0.0091

OVERALL MEANS AND STANDARD DEVIATIONS OF RHM

WEIGHT	MEAN=	97.78676	S.D.=	29.76735
LACTAT	MEAN=	1.38235	S.D.=	0.65613

COMBINED LEAST-SQUARES ANALYSIS OF VARIANCE

WEIGHT

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F	PROB
LOCATS	12	41660.44	3471.70	16.932	0.0000
HET %	3	1262.05	420.68	2.052	0.1104
REGRESSIONS					
AGE B LINEAR	1	20868.30	20868.30	01.776	0.0000
AGE B QUAD	1	8427.31	8427.31	41.100	0.0000
REMAINDER	118	24194.91	205.04		

LACTAT

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARE	F	PROB
LOCATS	12	10.79	0.89	3.407	0.0003
HET %	3	0.75	0.25	0.955	0.4166
REGRESSIONS					
AGE B LINEAR	1	13.79	13.79	52.225	0.0000
AGE B QUAD	1	4.07	4.07	15.426	0.0001
REMAINDER	118	31.16	0.26		

OVERALL MEANS AND STANDARD DEVIATIONS OF RHM

AGE MEAN= 5.59718 S.D.= 2.95058

COMBINED LEAST-SQUARES ANALYSIS OF VARIANCE

AGE

SOURCE	D.F.	SUM OF SQUARES	MEAN SQUARES	F	PROB
WEIGHT	151	1380.26	9.14	2.584	0.0000
IDH-2	2	10.01	5.00	1.416	0.2453
GPI-1	1	11.08	11.08	3.134	0.0784
SOD-1	2	25.56	12.78	3.613	0.0289
SEX	1	52.33	52.33	14.796	0.0002
LOCAT	15	140.71	9.38	2.652	0.0012
REMAINDER	182	643.77	3.53		

RHM NAME	ROW CODE	INDEPENDENT VARIABLE		NO. OBS.	EFFECTIVE NO.	CONSTANT ESTIMATE	STANDARD ERROR OF CONSTANT
AGE	1	MU		355	52.2	5.985	0.489
AGE	2	IDH-2	1	172	33.8	0.352	0.218
AGE	3	IDH-2	2	41	19.5	-0.439	0.295
AGE	0	IDH-2	3	142	36.8	0.087	0.209
AGE	4	GPI-1	2	326	52.4	-0.442	0.249
AGE	0	GPI-1	3	29	18.4	0.442	0.249
AGE	5	SOD-1	1	23	11.8	-0.473	0.406
AGE	6	SOD-1	2	251	47.2	-0.217	0.245
AGE	0	SOD-1	3	81	25.5	0.690	0.290
AGE	7	SEX	1	137	28.2	0.859	0.223
AGE	0	SEX	2	218	32.2	-0.859	0.223
AGE	8	LOCAT	11	4	2.3	-0.289	1.158
AGE	9	LOCAT	12	5	3.1	-0.346	1.000
AGE	10	LOCAT	14	7	4.8	-0.043	0.801
AGE	11	LOCAT	21	44	13.2	0.197	0.474
AGE	12	LOCAT	22	19	7.2	0.860	0.634
AGE	13	LOCAT	24	22	7.2	0.469	0.676
AGE	14	LOCAT	31	32	10.5	-0.520	0.539
AGE	15	LOCAT	32	33	11.0	1.762	0.524
AGE	16	LOCAT	33	40	13.8	-0.059	0.465
AGE	17	LOCAT	34	23	5.1	-0.635	0.813
AGE	18	LOCAT	41	52	13.7	-0.990	0.453
AGE	19	LOCAT	42	10	3.4	0.950	0.975
AGE	20	LOCAT	43	24	1.6	2.197	0.519
AGE	21	LOCAT	51	17	8.5	-1.010	0.602
AGE	22	LOCAT	52	9	5.2	-0.553	0.783
AGE	0	LOCAT	53	14	7.3	-1.986	0.663

APPENDIX 7.

Poacher detection probability on the Olympic Peninsula, WA

MPI	p = 0.913, q = 0.087	AA=.8336	Aa=.1589	aa=.0076
PGM-1	p = 0.813, q = 0.187	BB=.6610	Bb=.3041	bb=.0350

	AA	Aa	aa
BB	0.5513	0.1051	0.0050
Bb	0.2535	0.0483	0.0023
bb	0.0292	0.0056	0.0003

SOD-1	p = 0.383, q = 0.617	CC=.1467	Cc=.4726	cc=.3807
PEP-2	p = 0.905, q = 0.095	DD=.8190	Dd=.1720	dd=.0163

	CC	Cc	cc
DD	0.1201	0.3871	0.3118
Dd	0.0252	0.0813	0.0655
dd	0.0013	0.0043	0.0034

Listed above are the gene frequencies for four polymorphic enzyme loci on the Olympic Peninsula, WA. The matrices are the expected genotype frequencies for MPI and PGM-1 together, followed by those for SOD-1 and PEP-2. On the next page are the 81 possible genotype combinations for these loci, the expected frequency of each and the probability that two wapiti sampled at random on the Peninsula will share that genotype combination - simply the frequency squared. Because the probabilities for many of the genotypes are so small, they are listed in scientific notation, with the exponent in parenthesis. The sum of those probabilities, subtracted from 100%, gives the likelihood that two wapiti on the Olympic Peninsula, can be differentiated on the basis of polymorphism at those four loci.

GENOTYPE	FREQUENCY	PROBABILITY			
AABBCCDD	0.0662	4.4 (-3)	AABBccDd	0.0361	1.3 (-3)
AABbCCDD	0.0304	9.0 (-4)	AABbccDd	0.0166	2.7 (-4)
AAbbCCDD	0.0035	1.2 (-5)	AAbbccDd	0.0019	3.6 (-6)
AaBBCCDD	0.0126	1.6 (-4)	AaBBccDd	0.0069	4.7 (-5)
AaBbCCDD	0.0058	3.4 (-5)	AaBbccDd	0.0032	1.0 (-5)
AabbCCDD	0.0007	4.5 (-7)	AabbccDd	0.00037	1.4 (-7)
aaBBCCDD	0.0060	3.6 (-5)	aaBBccDd	0.00033	1.1 (-7)
aaBbCCDD	0.00028	7.8 (-8)	aaBbccDd	0.00015	2.2 (-8)
aabbCCDD	0.000036	1.3 (-9)	aabbccDd	0.00002	4.0 (-10)
AABBCcDD	0.2134	4.55 (-2)	AABBCCdd	0.00072	5.1 (-7)
AABbCcDD	0.0981	9.6 (-3)	AABbCCdd	0.00033	1.1 (-7)
AAbbCcDD	0.0113	1.3 (-4)	AAbbCCdd	0.000038	1.4 (-9)
AaBBCcDD	0.0407	1.6 (-3)	AaBBCCdd	0.00014	2.0 (-8)
AaBbCcDD	0.0186	3.5 (-4)	AaBbCCdd	0.000063	3.9 (-9)
AabbCcDD	0.0022	4.6 (-6)	AabbCCdd	0.0000072	5.3 (-11)
aaBBCcDD	0.0019	3.7 (-6)	aaBBCCdd	0.0000065	4.2 (-11)
aaBbCcDD	0.00089	7.9 (-7)	aaBbCCdd	0.0000053	2.8 (-11)
aabbCcDD	0.00012	1.4 (-8)	aabbCCdd	0.0000004	1.5 (-13)
AABBccDD	0.1719	2.95 (-2)	AABBCcdd	0.0024	5.7 (-6)
AABbccDD	0.0790	6.2 (-3)	AABbCcdd	0.0011	1.1 (-6)
AAbbccDD	0.0091	8.3 (-5)	AAbbCcdd	0.00012	1.6 (-8)
AaBBccDD	0.0327	1.1 (-3)	AaBBCcdd	0.00045	2.0 (-7)
AaBbccDD	0.0151	2.2 (-4)	AaBbCcdd	0.00021	4.3 (-8)
AabbccDD	0.0017	2.9 (-6)	AabbCcdd	0.000024	5.8 (-10)
aaBBccDD	0.0016	2.4 (-6)	aaBBCcdd	0.000021	4.6 (-10)
aaBbccDD	0.00072	5.2 (-7)	aaBbCcdd	0.0000099	9.8 (-11)
aabbccDD	0.000093	8.6 (-9)	aabbCcdd	0.0000013	1.7 (-12)
AABBCCDd	0.0139	1.9 (-4)	AABBccdd	0.0019	3.6 (-6)
AABbCCDd	0.0064	4.1 (-4)	AABbccdd	0.00086	7.4 (-7)
AAbbCCDd	0.00074	5.5 (-7)	AAbbccdd	0.00085	7.2 (-7)
AaBBCCDd	0.0026	6.8 (-6)	AaBBccdd	0.00036	1.3 (-7)
AaBbCCDd	0.0012	1.4 (-6)	AaBbccdd	0.00028	8.0 (-8)
AabbCCDd	0.00014	2.0 (-8)	Aabbccdd	0.000019	3.6 (-10)
aaBBCCDd	0.00013	1.6 (-8)	aaBBccdd	0.000017	2.9 (-10)
aaBbCCDd	0.000058	3.4 (-9)	aaBbccdd	0.0000078	6.1 (-11)
aabbCCDd	0.0000075	5.6 (-11)	aabbccdd	0.0000010	1.0 (-12)
AABBCcDd	0.0448	2.0 (-3)			
AABbCcDd	0.0206	4.1 (-4)	TOTAL	1.003	10.23 (-2)
AAbbCcDd	0.0023	5.6 (-6)			
AaBBCcDd	0.0085	7.3 (-5)		100.00%	
AaBbCcDd	0.0039	1.5 (-5)	-	10.23	
AabbCcDd	0.00046	2.1 (-7)			
aaBBCcDd	0.00041	1.7 (-7)		89.77%	Probability
aabbCcDd	0.00019	3.6 (-8)			
aabbCcDd	0.000024	5.8 (-10)			

Stalker selects stag for an estate guest to shoot.
Ghillie is at right.