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GENETIC VARIATION IN NINE NORTHERN SUBSPECIES OF THE COYOTE, *CANIS LATRANS*

A Thesis

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Degree

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Scott H. MacKenzie

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ABSTRACT

The range of Canis latrans extends from Alaska, south to Central America, and from California east to Nova Scotia. Within this area there are 20 described subspecies. Many of their current distributions were defined without consideration of gene flow. In this study, populations from nine different, contiguous northern subspecies of coyote were analyzed for allozyme variability, and genetic isolation. This was accomplished by comparing muscle tissue proteins electrophoretically on starch gels. A survey of 22 enzyme systems, consisting of 44 loci, was conducted on from 1 to 3 populations from each subspecies. Results of the study revealed that in most cases, the genetic similarity among populations of coyotes examined does not correspond to the subspecific designations described in the literature. Values of heterozygosity were consistent with that of other mammals and in contrast to some theoretical statements, this study presents another example of a large mammal which has average heterozygosity.

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I. INTRODUCTION

Biological systematics is the study of organismic diversity. There are two primary goals to any systematic investigation. The first is to determine the evolutionary relationships among taxa, and the second is to analyze and account for the variation within taxa (Simpson 1961; Mayr 1969). These two goals have arisen only recently in the history of taxonomy and systematics. Prior to 1859, the year that Charles Darwin published The Origin of Species, taxonomic theory was deeply rooted in the philosophy of ssentialism. This philosophy, based on the teachings of Plato and Aristotle, maintained that all objects (animate or inanimate) had a pure essence, or true nature (Simpson 1961; Mayr 1969; Sober 1987). Essentialism became the foundation of typological thinking that was pervasive in taxonomy. As taxonomists of that period searched for the essence of a species, they ignored the variation they observed, explaining that it was due to interference in development or the result of environmental interactions that prevented the organism from reaching its full potential. It was not until the latter part of the nineteenth century that Darwin recognized the importance of variation and it was realized that essence was an illusion (Sober 1987).

Between the mid-nineteenth and the mid-twentieth century, systematists searched for a definition of species.

As a result of that search, two primary definitions were proposed. The first definition was the biological species concept, proposed by Ernst Mayr (1940). He defined a species as groups of interbreeding populations that are reproductively isolated from other such groups. Simpson (1961) expanded on this idea by adding that the species is the unit of evolution and that any definition of species should encompass that concept. His definition was broad enough to contain Mayr's earlier biological species concept as a special case. Simpson (1961) defined an evolutionary species as "a lineage (ancestor-descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role." According to Simpson the role of a species is roughly equivalent to it's ecological niche.

The definition of subspecies presents more trouble. The species is the unit of evolution and is considered a natural taxonomic unit. The variation within a species is the raw material of change. As systematists began to move away from typological thinking, the geographic variation found within a species was no longer ignored. A subspecies is presently thought of as a collection of geographically isolated populations which differ from other subdivisions of a species (Mayr 1969).

A major problem with the concept of subspecies is the

lack of any consensus for what constitutes isolation, and how that isolation can be determined. Some authors have argued that the subspecies is too arbitrary, and should be abandoned (Wilson and Brown 1953). Other authors argue that the 75% rule should apply (Smith and White 1956; Simpson 1961; Mayr 1969). Under this rule, if a researcher is given organisms from two adjacent subspecies, he should be able to correctly place the organisms into their proper subspecies 75% of the time, based on available characters (Amadon 1949). Definition aside, there is little question that the concept of subspecies could be very important in clarifying the biogeographic history of a species, if it reflects a barrier to gene flow.

Recently, the field of systematics has been greatly influenced by biochemical techniques, particularly by starch gel electrophoresis. Until twenty years ago, morphological or behavioral characters were the primary critera used to determine phylogenetic relationships. During the last two decades, several authors have assessed the relative value of biochemical and morphological methods in systematics (Avise 1975; Buth 1984; Hillis 1987). While there are advantages and disadvantages in both data sets, the concensus opinion is that, for living organisms, allozyme technology is sufficiently advanced to generally offer a more objective, precise and parsimonious method

for determing phylogenies than traditional morphological techniques (Avise 1975 and Buth 1984). Hillis (1987), on the other hand, states that in order for systematic studies to be most valuable, a combination of both morphological and molecular data sets will lead to the best description of phylogenetic relationships. Researchers should be aware that the value of the electrophoretic technique can be over-estimated. Inherent sources of error in this method are the potentially biased sample of genes examined and an inherent underestimate of existing variability due to non-detectable amino acid substitutions. These problems are more likely to arise when making comparisons among taxa such as congeneric species or confamilial genera (Avise 1975). The limitations are not as severe when comparing conspecific populations, primarily because of the short time since divergence between the populations.

Comparisons of genetic variability among subspecies is wide-spread in the recent literature. For mammals, most of these studies have been restricted to several different rodent genera (e.g. Peromyscus: Selander et al. 1971; Smith et al. 1973; Avise et al. 1974; Kilpatrick and Zimmerman 1976; Rennert and Kilpatrick 1987; Calhoun et al. 1988, Sigmodon Johnson et al. 1972, Dipodomys: Hamilton et al. 1987, Geomys: Sudman et al. 1987, Cynomys: Chesser 1983; McCullough and Chesser 1987, and Megadontomys: Werbitsky

and Kilpatrick 1987). Subspecific studies appear to have focused primarily on rodents perhaps, in part, because of the large number of different taxa, and the relative ease with which large sample sizes can be obtained. It is also widely assumed that larger mammals have a low degree of genetic variability relative to small ones (Selander and Kaufman 1973; Allendorf et al. 1979; Nevo 1978; Wooten and Smith 1985). On the other hand, Simon and Archie (1985) have observed that heterozygosity estimates depend more on the laboratory doing the study than on the type of organism or the number of loci considered. Recent studies on Indian rhinoceros, Rhinoceros unicornis (McCracken, pers. comm.), wild boars, Sus scrofa ferus (Hartl and Csaikl 1987), black bear, Ursus americanus (Wathen et al. 1985), and assorted artiodactyl species (Baccus et al. 1983; Ramsey et al. 1979) demonstrate that some large mammals do have variability similar to that of rodents.

Prior to this study, there were no molecular, subspecific studies on canids with the exception of Hamilton (1984), which examined allozyme differences between coyote populations in Tennessee. The majority of allozyme studies on canids have dealt with differences at the family level (Clark et al. 1975; Simonsen 1976; Wayne and O'Brien 1987), or with differences between species within the genus, Canis (Fisher et al. 1976; Cole et al.

1977; Ferrell et al. 1980). These studies have shown that, as a rule, the genus has relatively low variability.

In this study, I use starch gel electrophoresis to analyze subspecific differences within coyotes, Canis latrans and to further test the hypothesis that large mobile mammals have reduced genetic variability relative to other mammals.

Coyotes are medium sized predators of the order Carnivora. They are typical members of the family Canidae, which includes animals such as the fox, jackal and wolf. Like other members of the genus Canis, the coyote has a thin body, long narrow muzzle, erect ears, bushy tail and long legs adapted for running (Kurten 1980; Andrews and Boggess 1978). They are similar in appearance to a small german shepard dog. The head and body length of an adult coyote ranges between 70 and 97 centimeters (Bowen 1984). Their body weight is between 9.5 kilograms, in western populations, and 18-20 kilograms in northeastern populations (Andrews and Boggess 1978; Hilton 1978; Henshaw 1982). Generally, the pelage is dorsally gray, with occasional flecks of black scattered throughout. The dorsal coloration gradually blends into a light cream on the ventral surface (Caturano and Harrison 1981).

The coyote is the smallest of the three species of wild Canis living in North America. Since the end of the

19th century, the coyote has experienced a rapid and extensive range expansion (Nowak 1979; Gipson 1978; Hilton 1978). Before this time, coyotes were confined to the open prairies and arid regions of North America (Young and Jackson 1951; Nowak 1979). However, coyotes now can be found in moist deciduous forests in much of eastern and western North America, areas from which they were previously absent (Messier and Barrette 1982; Caturano and Harrison 1981). These new habitats may also represent environmental clines (Endler 1977), across which little genetic exchange between populations would be expected.

In all parts of its range, the coyote has proved to be an opportunistic feeder. The bulk of the coyote's diet is meat, primarily rodents and leporids, but they will eat a wide range of other foods, including: livestock (cattle, sheep and pigs), birds, reptiles, various invertebrates, berries and grasses (Ortega 1987; Van Vuren and Thompson 1982; Andrews and Boggess 1978; Berg and Chesness 1978; Hilton 1978; Ozaga and Harger 1966; Fichter et al. 1955). The diet of coyotes varies seasonally, particularly in the northern part of it's range. In winter, when their usual foods are scarce, the coyote's diet consists mostly of deer (Odocoileus virginianus) or caribou (Rangifer caribou) carrion (Ozaga and Harger 1966; Bekoff and Wells 1981). As a result of taking larger prey, coyotes

switch, in winter, from foraging alone or in pairs to hunting and defending food resources in packs of from three to eight animals. When spring returns, there is an increase in the rodent populations and greater dispersion of the resource base. This causes the winter packs to fragment, usually into pair-bonded males and females. Pairs then search for suitable den sites (Bekoff and Wells 1981).

Coyotes are monoestrus, usually coming into season between January and March. An exception is the New England coyote (C. l. variant.), in which females reach sexual maturity in their second year (Hilton 1978; Silver and Silver 1969). Typically females remain in heat for five days. The gestation period lasts about 65 days. In early spring, five to eight altrical pups are born and are dependent on the female for several weeks. During this period, the male will provision the female with food, and guards the pups while the female is hunting (Hilton 1978; Nowak 1979; Harrison and Gilbert 1985).

Coyotes typically disperse from their natal range between the ages of 9 to 24 months (Berg and Chesness 1978; Hilton 1978; Andelt 1985). This correlates well with the age of maturity of these animals, however dispersal is also influenced in part by the degree of habitat saturation (Messier and Barrette 1982; Andelt 1985; Bekoff and Wells 1986).

Coyotes have great vagility, and individuals have been reported to disperse up to 128 kilometers (Ozaga and Harger 1966), though the average distance traveled in their first year is closer to 40 kilometers (Andrews and Boggess 1978; Berg and Chesness 1978; Bowen 1982; Woodruff and Keller 1982). Some authors (Berg and Chesness 1978; Woodruff and Keller 1982; Andelt 1985) report that males emigrate the greater distance, while others (Nellis and Keith 1976; Andrews and Boggess 1978; Bowen 1982) claim that females are more far-ranging. Although there is some disagreement as to which gender disperses further, the consensus is that males are more likly to disperse than are females. This hypothesis is supported by the high percentage of males found in newly established populations in Nova Scotia and New Brunswick (Moore and Millar 1984).

Their sociality, flexibility in diet, high fecundity and dispersal abilities, coupled with the removal of their chief competitors (Canis lupus and Canis rufus) from much of their former range, all have contributed to the coyote's spread over most of North America (Gipson 1978; Hilton 1978; Henshaw 1982). The range of the coyote now extends from Alaska south to Honduras, and from California east to Nova Scotia (Fig.1) Within this area, there are twenty described subspecies (Hall 1981; Nowak 1979). With the exception of C.l. variant(the New England canid)

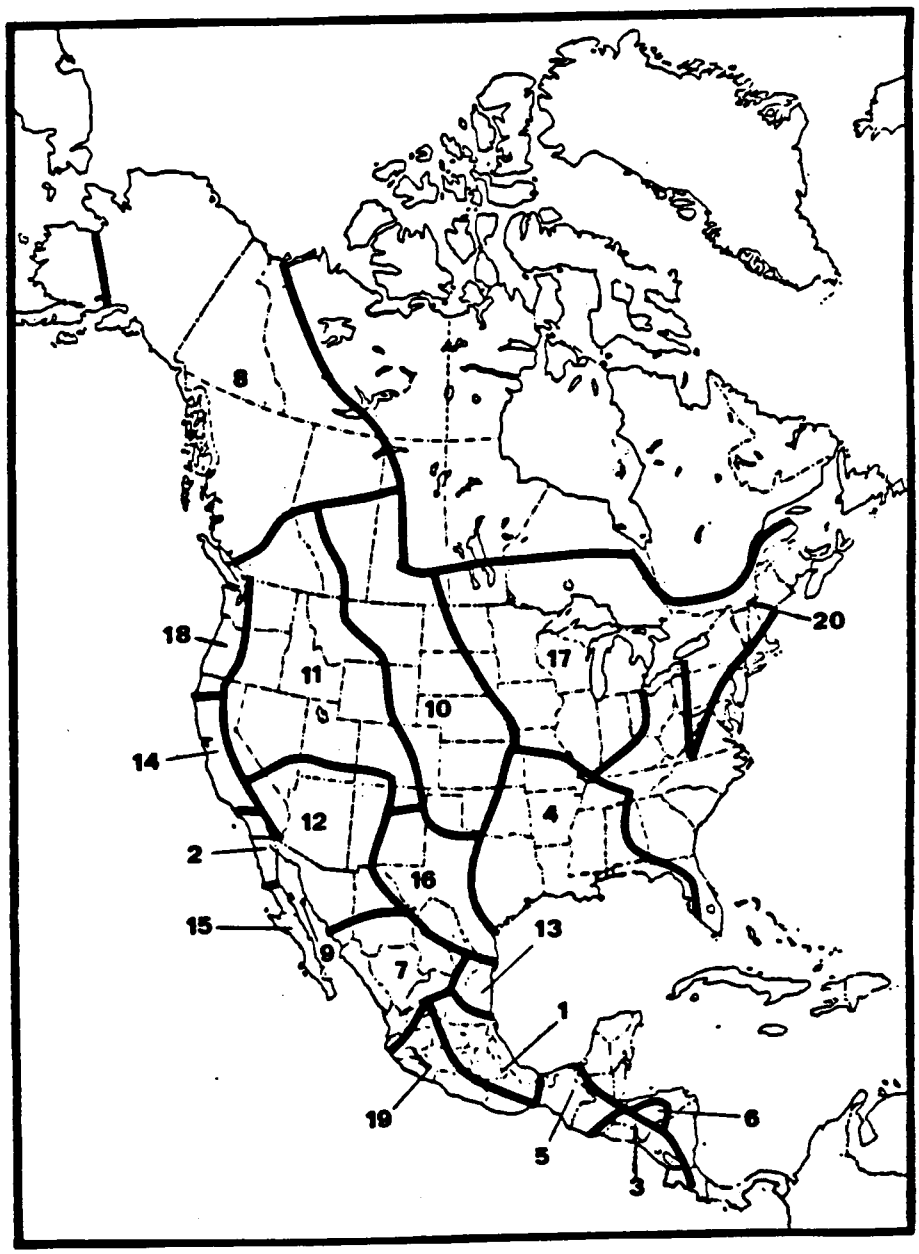

Figure 1. Distribution of the twenty subspecies of coyote, Canis latrans. 1) C.l.cagottis, 2) C.l. clepticus, 3) C.l. dickeyi, 4) C.l. frustror, 5) C.l. goldmani, 6) C.l. hondurensis, 7) C.l. impavidus, 8) C.l. incolatus, 9) C.l. jamesi, 10) C.l. latrans, 11) C.l. lestes, 12) C.l. mearnsi, 13) C.l. microdon, 14) C.l. ochropus, 15) C.l. peninsulae 16) C.l. texensis, 17) C.l thamnos, 18) C.l. umpquensis, 19) C.l. vigilis, 20) C.l. variant (Hall 1981; Nowak 1979).

described by Lawrence and Bossert (1975), all subspecies of coyote were described between the early nineteenth and mid-twentieth century. All of these subspecies were described from morphological characters. Some of the descriptions are based on from one to only a few specimens (Say 1823; Townsend 1912; Young and Jackson 1951). It is hoped that this analysis of protein variability will provide a clearer understanding of the status of the subspecies of coyote.

II. METHODS

Specimens (n=154) of coyotes, from 14 populations, representing 9 subspecies were collected for genetic analysis. (Table 1 and Fig.2). Subspecific designation follows Hall (1981) and Nowak (1979). Coyotes were collected by professional trappers between November and March of 1987 and 1988. Information on weight, gender and approximate age of the animals was recorded, as were the county and state of collection. Only animals that had been dead for less than twelve hours were used in this study. Trappers removed approximately 20-30 grams of muscle tissue from the gluteal region of each animal. Tissue was numbered, dated, wrapped in foil or plastic wrap, and frozen to ~-20°C. None of the tissue samples were stored at this temperature for longer than three weeks. When 5-20 samples had been collected from an area, the tissue was shipped in a styrofoam box and received in the laboratory the next day. The tissue then was inspected, catalogued, and frozen to -80°C.

Subsamples of 1.5 grams of tissue from each animal were allowed to thaw on ice and were then minced with a razor blade. The sample then was placed into a centrifuge tube and one millilitre of ice cold grinding buffer (10mM Tris-HCl, 1mM EDTA, 1mM NADPH pH 6.7). The mixture was

Table 1. Subspecies, population designations, collection locales and sample sizes of *Canis latrans* included in this study.

Subspecies	Population	Locality	N
C.l. incolatus	AK-1	Copper Center, AK	11
C.l. lestes	CA-3	Sierra and Plumas Co., CA	6
	ID-1	Gem Co., ID	5
	MT-1	Park Co, MT	20
C.l. ochropus	CA-1	Kern and Santa Barbara Co., CA	10
	CA-2	Napa and Solano Co., CA	4
C.l. clepticus	CA-4	San Diego Co., CA	10
C.l. latrans	NE-1	Lincoln Co., NE	10
C.l. texensis	TX-2	Duval Co., TX	5
C.l. frustror	OK-1	Muskogee and Wagoner Co., OK	9
	TX-1	Jefferson, Liberty and Hamilton Co., TX	17
C.l. thamnos	MO-1	Lincoln Co., MO	9
	MI-1	Ogemaw, Oscoda, Kalakaska, Crawford and Ostego Co., MI	18
C.l. var.	ME-1	Hancock, Penobscott and Washington Co., ME	17

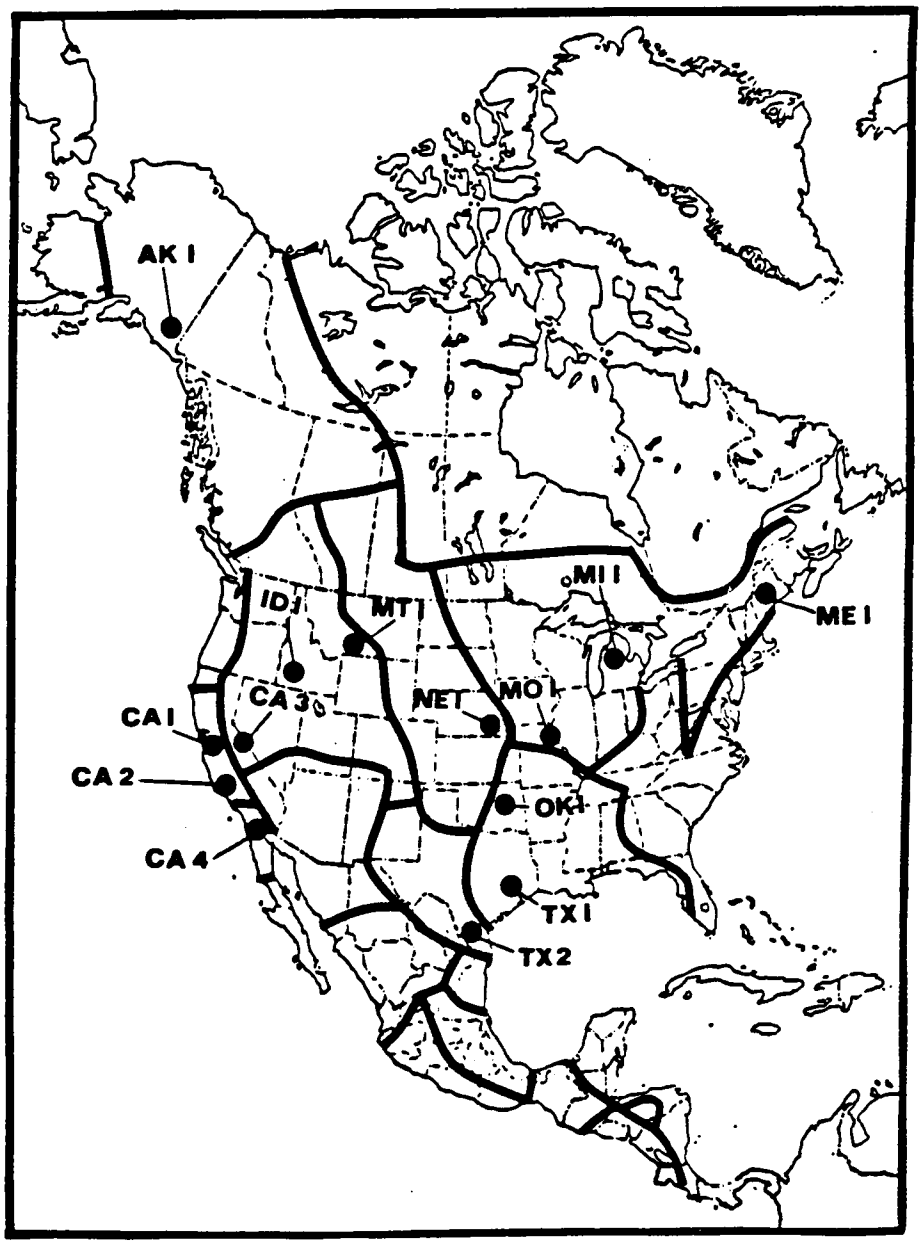

Figure 2. Collection site of Canis latrans used in this study. See Table 1 for abbreviations and Figure 1 for identities of subspecies.

vortexed vigorously for 30 seconds and then incubated on ice for 90 minutes. The mixture then was vortexed again for 30 seconds and then centrifuged at 13,000 rpm for 45 minutes. The supernatant was decanted to a new tube, and frozen to -80°C.

An initial survey of 43 enzymes using seven different buffer systems was completed using samples from Maine, Nebraska, and Tennessee. Starch gel electrophoresis was carried out as described elsewhere (Harris and Hopkinson 1976; Selander et al. 1971). Of the 43 enzyme systems surveyed, 22 gave consistently scoreable results. Five buffer systems were used to resolve these enzymes and a total of 44 presumptive genetic loci were available for consideration. Enzyme loci and buffer systems used in this study are listed in Table 2. Buffer recipes are provided in the Appendix.

Genetic loci and alleles were scored by their relative migraton rates. In the case of a multiple locus enzyme, the most anodal migrating locus was designated as "1", the next "2", until all loci were identified. Cathodal migrating loci were considered the slowest loci, and are recorded as such. Alleles were designated alphabetically according to migration rate; "A" designating the furthest migrating allele observed, "B" the next, etc.. A locus was considered polymorphic if the frequency of the most common allele was

Table 2. Protein loci, gene symbols, E.C. number (McAlpine et al. 1985), and buffer systems used in this study.

	LOCUS	E.C. NUMBER	GENE SYMBOL	BUFFER
1.	Acontinase 1	4.2.1.3	ACO1	TC 8.0
2.	Adenylate kinase 1	2.7.4.3	AK1	Poulik
3.	Adenylate kinase 2	2.7.4.3	AK2	Poulik
4.	Adenylate kinase 3	2.7.4.10	AK3	Poulik
5.	Adenylate kinase 4		AK4	Poulik
6.	Aldolase 1	4.1.2.13	ALDOA1	TM 7.4
7.	Aldolase 2	4.1.2.13	ALDOA2	TM 7.4
8.	Creatine kinase 1	2.7.3.2	CKMM1	TM 7.4
9.	Creatine kinase 2	2.7.3.2	CKMM2	TM 7.4
10.	Esterase 1	3.1.1.1	EST1	TC 6.7
11.	Esterase 2	3.1.1.1	EST2	TC 6.7
12.	Esterase 3	3.1.1.1	EST3	TC 6.7
13.	Fumerte hydratase 1	4.2.1.2	FH1	TM 7.4
14.	Fumerte hydratase 2	4.2.1.2	FH2	TM 7.4
15.	General Protein 1		GP1	TVB
16.	General Protein 2		GP2	TVB
17.	General Protein 3		GP3	TVB
18.	Glutamate oxaloacetate transaminase 1	2.6.1.1	GOT1	TC 6.7
19.	Glutamate oxaloacetate transaminase 2	2.6.1.1	GOT2	TC 6.7
20.	alpha-glycerophodphate dehydrogenase 1	1.1.1.8	GPD1	TVB
21.	alpha glycerophosphate dehydrogenase 2	1.1.1.8	GPD2	TVB
22.	alpha glycerophosphate dehydrogenase 3	1.1.1.8	GPD3	TVB
23.	Glucose phosphate isomerase	5.3.1.9	GPI	TM 7.4
24.	Glyceraldehyde-3 phosphate dehydrogenase	1.2.1.12	GAPD	TC 8.0
25.	Glucose-6-phosphate deydrogenase	1.1.1.49	G6PD	TVB
26.	Hexokinase 1	2.7.1.1	HK1	TC 8.0
27.	Hexokinase 2	2.7.1.1	HK2	TC 8.0
28.	Hexokinase 3	2.7.1.1	HK3	TC 8.0
29.	Leucine amino peptidase	3.4.1.1	LAP	Poulik
30.	Lactose dehydrogenase A	1.1.1.27	LDHA	Poulik
31.	Lactose dehydrogenase B	1.1.1.27	LDHB	Poulik
32.	Malate dehydrogenase 1	1.1.1.37	MDH1	TC 8.0
33.	Malate dehydrogenase 2	1.1.1.37	MDH2	TC 8.0
34.	Malic enzyme 1	1.1.1.40	ME1	TC 6.7

Table 2 (continued)

	ENZYME	E.C. NUMBER	GENE SYMBOL	BUFFER
35.	Malic enzyme 2	1.1.1.40	ME2	TC 6.7
36.	Mannosephosphate isomerase 1	5.3.1.8	MPI1	TC 8.0
37.	Mannosephosphate isomerase 2	5.3.1.8	MPI2	TC 8.0
38.	Octanol dehydrogenase 1	1.1.1.1	ODH1	TM 7.4
39.	Octanol dehydrogenase 2	1.1.1.1	ODH2	TM 7.4
40.	6-phosphogluconate dehydrogenase 1	1.1.1.43	PGD1	TC 8.0
41.	6-phosphogluconate dehydrogenase 2	1.1.1.43	PGD2	TC 8.0
42.	Phosphoglucomutase	2.7.5.1	PGM1	Poulik
43.	Superoxide dismutase 1	1.15.1.1	SOD1	TVB
44.	Superoxide dismutase 2	1.15.1.1	SOD2	TVB

less than 0.99 across the species. Data analysis was conducted on the BIOSYS-1 program (Swofford and Selander 1981). Pair-wise comparisons were made using Rogers's coefficient of similarity, "S" (Rogers 1972) and Nei's genetic identity, "I" (Nei 1978). Phenograms, based on Rogers's similarity were constructed using Sokal and Sneath's (1963) algorithm, UPGMA (Unweighted pair-group methods using arithmetic means). The combination of these methods has been shown to produce phylogenetic trees that currently are most accurate, when 50 to 75 loci are examined (Nei et al. 1983; Rohlf and Wooten 1988). Though a rate-independent model using an out-group may have added to the information obtained from this study, (Hillis 1987) the absence of an out-group rendered accurate Wagner tree construction impossible.

III. RESULTS

Of the 44 loci examined, 21 were polymorphic (AK1, AK3, AK4, ALDOA2, EST1, EST2, EST3, GOT1, GPD2, GPD3, GPI, G6PD, HK1, HK2, LDHA, MDH1, MDH2, ME1, MPI1, ODH2, and PGD1) and 21 were monomorphic (ACO1, AK2, ALDOA1, CKMM1, CKMM2, FH1, FH2, GOT2, GP1, GP3, GPD1, GAPD, HK3, LAP1, LDHB, ME2, MPI2, ODH1, PGM1, PGD2, and SOD2). Two loci, GP2 and SOD1, were monogenic within all populations but not all populations were fixed for the same allele. All populations shared the same common allele at 16 of the polymorphic loci. When the same allele was not common to all populations, it was generally the California populations (CA-1, CA-2, CA-3, CA-4) which were deviant. Fixed allelic differences between populations occurred at the SOD1, GP2, MDH1 and GPD3 loci (Table 3).

Mean observed individual heterozygosity values (H_O) ranged from 0.006 for individuals in TX-2 to 0.062 for those in CA-4. Mean heterozygosity over all populations was 0.034 ± 0.017. The percent of loci polymorphic per population (P) also ranged widely between populations. Values ranged from P = 4.55% for NE-1 and TX-2, to P = 27.27% for CA-4. Over all populations, the mean proportion of loci polymorphic was 14.93% (Table 4). For this statistic, a locus was considered polymorphic when the

Table 3. Allelic frequencies for 23 polymorphic loci in 14 populations of Canis latrans.

Locus	allele	CA-4	CA-1	CA-2	CA-3	ID-1	MT-1	AK-1	NE-1	MO-1	MI-1	ME-1	OK-1	TX-1	TX-2
AK1	A'	0.15	-	-	-	-	-	-	-	0.06	-	-	-	-	-
	A	0.85	-	-	-	-	0.17	-	0.40	-	0.47	0.27	-	-	-
	B	-	1.00	1.00	1.00	1.00	0.83	1.00	0.60	0.94	0.53	0.73	1.00	1.00	1.00
AK3	A	-	0.50	0.83	0.30	-	-	0.32	-	-	0.06	0.11	0.39	-	-
	B	0.67	0.50	0.17	0.70	1.00	1.00	0.68	1.00	1.00	0.88	0.85	0.61	1.00	1.00
	C	0.33	-	-	-	-	-	-	-	-	0.06	0.04	-	-	-
AK4	A	0.30	-	-	-	-	-	-	-	-	-	-	-	0.06	-
	B	0.70	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.94	1.00
ALDOA2	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	1.00
	C	-	-	-	-	-	-	-	-	-	-	-	-	0.11	-
EST1	A'	-	-	-	-	-	0.03	-	-	-	0.03	-	-	-	-
	A	0.94	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00
	B	0.06	-	-	-	-	0.03	-	-	-	0.03	-	-	-	-
EST2	A	0.50	0.70	1.00	1.00	0.90	0.87	0.80	1.00	0.81	0.96	0.79	0.61	0.80	0.87
	B	0.50	0.30	-	-	0.10	0.13	0.20	-	0.19	0.04	0.21	0.39	0.20	0.13
EST3	B	0.90	0.85	0.75	0.67	0.90	1.00	0.96	1.00	0.89	0.89	1.00	1.00	0.63	1.00
	C	0.10	0.15	0.25	0.33	0.10	-	0.04	-	0.11	0.11	-	-	0.37	-
GDT1	B	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00	0.88	0.94	0.94	1.00
	C	0.05	-	-	-	-	-	-	-	0.17	-	0.12	0.06	0.06	-
GP2	A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
	B	-	-	-	-	-	-	-	1.00	-	-	-	-	-	
GPD2	A	0.25	0.35	0.33	0.42	-	-	-	-	-	-	-	-	-	-
	B	0.75	0.65	0.67	0.58	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPD3	B	1.00	0.15	-	-	1.00	1.00	0.91	1.00	1.00	1.00	1.00	0.78	1.00	1.00
	C	-	0.85	1.00	1.00	-	-	0.09	-	-	-	-	0.22	-	-
GPI	A'	-	-	0.25	0.25	0.10	-	-	-	-	0.03	-	-	0.14	-
	A	0.31	0.06	-	0.08	-	0.05	0.41	-	-	0.03	0.41	0.06	0.03	-
	B	0.69	0.94	0.75	0.67	0.90	0.95	0.59	1.00	1.00	0.94	0.59	0.94	0.83	1.00
G6PD	A	0.22	0.67	-	-	-	-	0.23	-	-	-	-	-	-	
	B	0.78	0.33	1.00	1.00	1.00	1.00	0.77	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HK1	A	-	-	-	-	0.80	-	-	-	-	-	-	-	-	-
	B	1.00	1.00	1.00	1.00	0.20	1.00	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	-	-	-	-	-	-	0.09	-	-	-	-	-	-	-
HK2	A	-	-	0.13	-	-	-	-	-	-	-	-	-	-	-
	B	0.75	1.00	0.87	1.00	1.00	1.00	0.95	1.00	1.00	0.86	0.73	0.89	1.00	0.50
	C	0.25	-	-	-	-	-	0.05	-	-	0.14	0.27	0.11	-	0.50
LDH1	B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	0.94	1.00	1.00	1.00	1.00	1.00
	C	-	-	-	-	-	-	-	0.05	0.06	-	-	-	-	-
MDH1	A	-	0.45	1.00	1.00	-	-	-	-	-	0.08	-	-	0.06	-
	B	0.80	0.55	-	-	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	0.94	1.00
	C	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-
MDH2	A	0.10	-	-	-	-	-	-	-	-	0.11	0.06	-	-	-
	B	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.89	0.94	1.00	1.00	1.00
ME1	A	-	-	-	-	0.10	-	-	-	-	0.06	0.21	-	0.06	-
	B	1.00	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	0.94	0.79	1.00	0.94	1.00

Table 3 (continued)

Locus	allele	CA-4	CA-1	CA-2	CA-3	ID-1	MT-1	AK-1	NE-1	MO-1	MI-1	ME-1	OK-1	TX-1	TX-2
MPI1	A	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-
	B	1.00	1.00	1.00	1.00	0.30	0.97	0.59	1.00	0.94	1.00	0.76	0.89	1.00	1.00
	C	-	-	-	-	0.70	0.03	0.41	-	-	-	0.24	0.11	-	-
ODH2	A	-	0.80	1.00	-	-	-	-	-	-	-	-	-	-	-
	B	1.00	0.15	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	C	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-
PGD1	A	-	-	-	-	-	-	0.36	-	-	-	-	0.06	-	-
	B	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00	1.00	1.00	0.94	1.00	1.00
SOD1	A	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-
	B	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	1.00
	C	-	-	-	-	1.00	1.00	1.00	-	1.00	1.00	-	1.00	1.00	-
	D	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-

Table 4. Mean heterozygosity (H) values and mean percent of loci polymorphic (P), of coyotes.

Population	P (%)	H expected	H direct-count
CA-4	27.3	0.084	0.062
CA-1	22.7	0.074	0.061
CA-2	13.6	0.049	0.019
CA-3	9.1	0.042	0.042
ID-1	13.6	0.033	0.032
MT-1	6.8	0.017	0.017
AK-1	18.2	0.069	0.046
NE-1	4.5	0.013	0.016
MO-1	15.9	0.027	0.026
MI-1	18.2	0.042	0.033
ME-1	18.2	0.054	0.052
OK-1	18.2	0.046	0.033
TX-1	18.2	0.039	0.036
TX-2	4.5	0.016	0.006

frequency of the common allele within a population was < 0.99. Therefore, the GP2 and SOD1 loci, which showed variation among populations but were monogenic within populations, did not contribute to this estimate of "P".

In five populations (AK-1, CA-1, CA-4, ME-1, MI-1 and OK-1) genotype frequencies at one or more polymorphic locus differed significantly from Hardy-Weinberg expectations (Table 5). At all loci, the deviation resulted from a deficiency or absence of heterozygotes at the locus under examination. Significant deviation at the 0.05 level was determined using the G^2-test for goodness-of-fit (Sokal and Rohlf 1981), in which it was necessary to pool individuals that were either homozygotes or heterozygotes. G^2-tests used Williams's correction (Sokal and Rohlf 1981) for a more conservative test.

Both Nei's (1978) genetic identity (I) and Rogers's (1972) genetic similarity (S) were calculated between all pairs of populations. Values for I ranged between 0.846 and 0.998. S values were between 0.818 and 0.984 (Table 6). Populations from California diverged the most from the other populations, having a mean S value of 0.876 $\pm$ 0.03 (range 0.818 to 0.919) when compared with groups from outside California. The mean value of S between all pairs of the four California populations was 0.911. The lowest S value recorded between populations, outside of California

Table 5. Loci in coyoye populations that deviated significantly from the Hardy-Weinberg equilibrium.

Population	Locus	G^2	d.f.	p<
CA-1	G6PD	7.77	1	0.01
CA-4	GPI	3.87	1	0.05
	HK2	7.69	1	0.01
	MDH1	7.82	1	0.01
AK-1	MPI1	8.40	1	0.01
	PGD1	4.21	1	0.05
MI-1	MDH2	7.96	1	0.01
ME-1	GOT1	7.95	1	0.01
OK-1	EST2	5.96	1	0.02
	GPD3	7.77	1	0.01

Table 6. Matrix of genetic similarity, "S", (Rogers 1972) and genetic identity, "I", (Nei 1978) for coyotes. S values are above the diagonal, I values are below.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. CA-1	*****	0.951	0.923	0.890	0.849	0.878	0.872	0.877	0.871	0.863	0.846	0.892	0.878	0.871
2. CA-2	0.991	*****	0.942	0.860	0.818	0.844	0.842	0.842	0.846	0.830	0.819	0.855	0.849	0.844
3. CA-3	0.960	0.964	*****	0.903	0.867	0.893	0.880	0.891	0.889	0.873	0.868	0.895	0.901	0.887
4. CA-4	0.940	0.901	0.944	*****	0.887	0.924	0.903	0.919	0.925	0.921	0.892	0.922	0.919	0.921
5. ID-1	0.890	0.851	0.897	0.938	*****	0.954	0.938	0.952	0.940	0.920	0.901	0.936	0.949	0.924
6. MT-1	0.916	0.876	0.920	0.966	0.975	*****	0.950	0.984	0.975	0.948	0.942	0.968	0.974	0.960
7. AK-1	0.921	0.886	0.918	0.959	0.977	0.988	*****	0.947	0.935	0.921	0.894	0.958	0.942	0.921
8. MO-1	0.916	0.876	0.921	0.966	0.976	0.999	0.987	*****	0.966	0.945	0.933	0.965	0.976	0.954
9. MI-1	0.912	0.879	0.921	0.964	0.969	0.997	0.982	0.972	*****	0.946	0.935	0.954	0.962	0.944
10. ME-1	0.912	0.874	0.914	0.966	0.953	0.973	0.964	0.972	0.973	*****	0.922	0.940	0.933	0.947
11. NE-1	0.887	0.850	0.894	0.937	0.925	0.953	0.935	0.950	0.953	0.948	*****	0.913	0.920	0.953
12. OK-1	0.929	0.894	0.928	0.967	0.972	0.994	0.993	0.995	0.989	0.971	0.942	*****	0.958	0.942
13. TX-1	0.916	0.879	0.926	0.965	0.992	0.996	0.985	0.998	0.992	0.968	0.945	0.992	*****	0.944
14. TX-2	0.911	0.874	0.916	0.965	0.947	0.972	0.959	0.972	0.969	0.972	0.969	0.969	0.968	*****

was 0.894, between AK-1 and NE-1. Conversely, most closely related populations were MO-1 and MT-1 with S = 0.984.

The UPGMA phenogram generated from the Rogers's genetic similarity matrix (Fig. 3) was chosen over the phenogram generated using genetic identity because the value of its percent deviation, 1.47, (Fitch and Margoliash 1967) was the lower.

Six major "clusters" can be identified from the phenogram (Fig. 3). Three of the four California populations (CA-1, CA-2, and CA-3) formed a cluster that was rooted at S = 0.865. A fourth California population (CA-4) separated from all remaining populations at S = 0.911. This fourth California population clustered closely with other California populations but showed higher similarity to TX-2 and NE-1. TX-2 and NE-1 , or south-central coyotes, branch off at 0.920, and cluster at 0.955. ME-1 branches off by itself at 0.940. ID-1 and AK-1 form an unresolved branch at 0.945, but are most similar to a north-east cluster of populations. The north-east plains cluster is the largest group of populations, consisting of OK-1, MO-1, MT-1, TX-1 and MI-1, and representing three subspecies (C.l. thamnos, C.l frustror and C.l. lestes). This cluster is rooted at 0.960. Geographic representation of the major clusters can be seen in Figure 4. For the

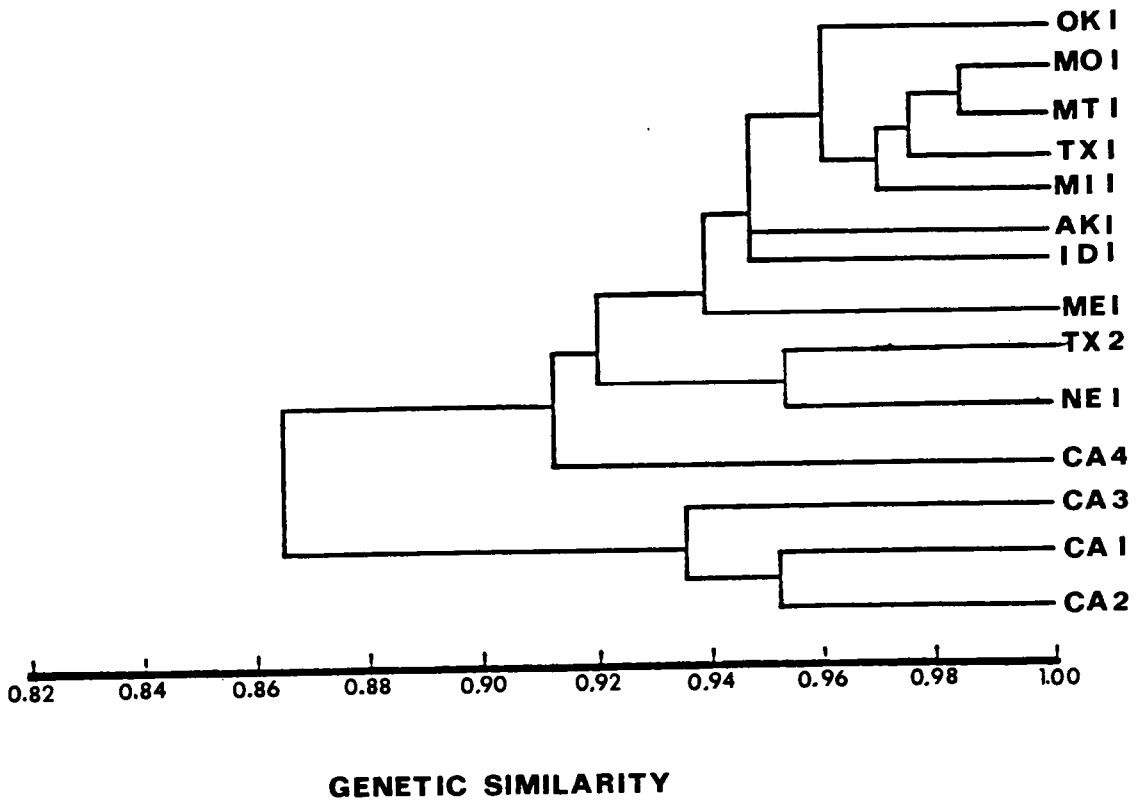

Figure 3. Phenogram of coyote subspecific similarities. Generated from Rogers's (1972) similarity coefficient, and clustered using UPGMA.

purposes of this diagram, distinct "clusters" consist of a population or group of populations for which similarity is > 0.930 as determined from the UPGMA phenogram (Fig. 3).

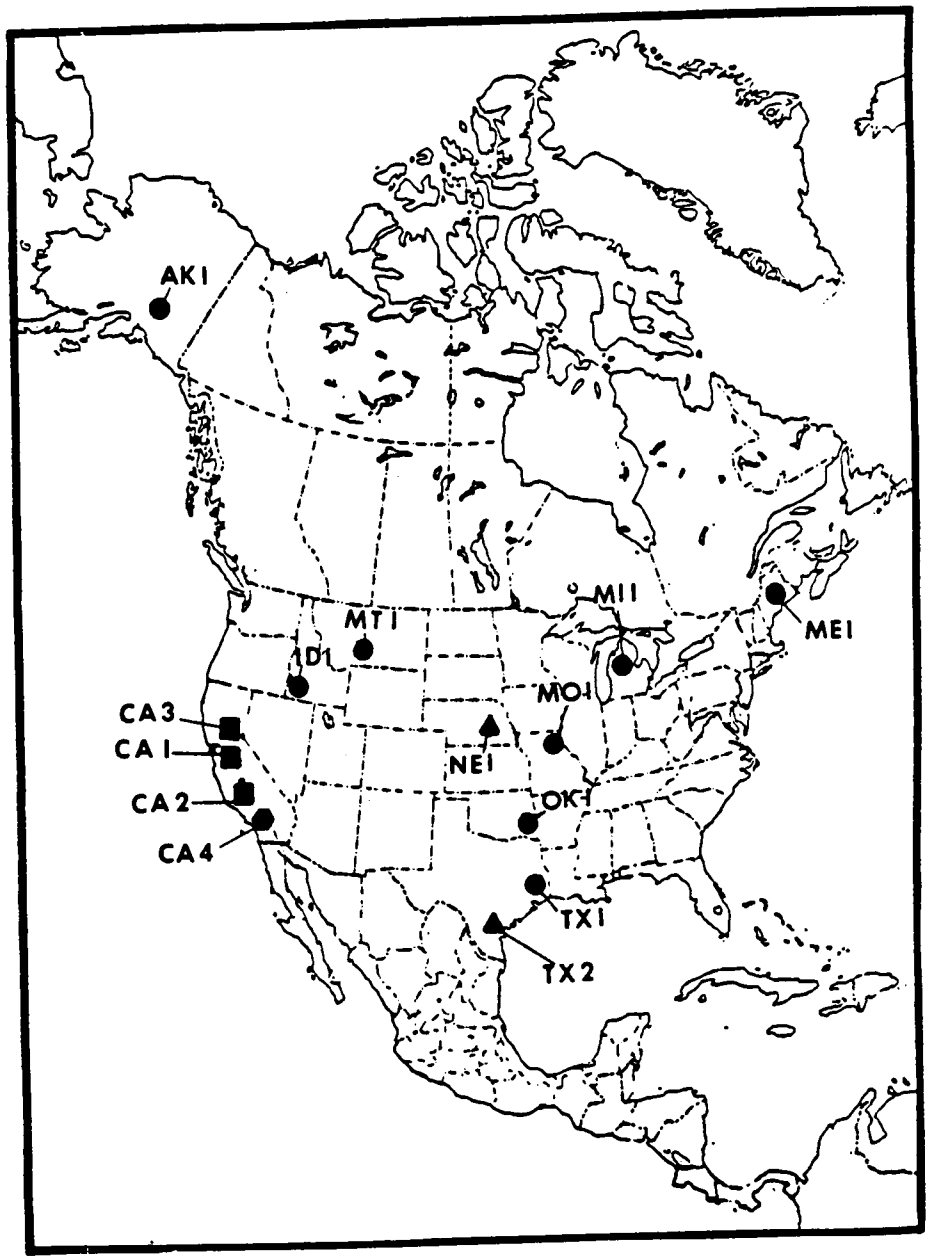

Figure 4. Map of coyote populations "clustering" at a genetic similarity of greater than 0.930. as observed from the UPGMA phenogram (Fig. 3). Common symbol denotes relatedness according to 0.930 criteria.

IV. DISCUSSION

Current Taxonomy and Genetic Variation Between Populations

Genetic similarity values for a variety of organisms, both invertebrates and vertebrates, provide a guideline for the degree of genetic differentiation expected at different taxonomic levels. Overall, populations of "lower" vertebrates generally have greater genetic similarity values than do populations of "higher" vertebrates (Ayala 1982; Ayala 1975; Avise 1975). For mammals, local populations were observed to have genetic similarity values of S = 0.944, (range 0.90 - 1.00). For different subspecies mean S was 0.781 (range 0.769 - 0.793). For species within the same genus, S was 0.719 (range 0.61 - 0.85) (Ayala 1982; Ayala 1975; Avise 1975). Subsequent systematic studies on mammals generally have yielded similarity data that fall within the range of the average values listed above (Sudman et al. 1987; Filippucci et al. 1987; Rennert and Kilpatrick 1987).

The data presented in this study demonstrate that, in most cases, the genetic similarity among the populations of coyotes examined do not correspond to the subspecific

designations described in the literature. Values of genetic similarity between populations from widely separated geographic areas often were very high (e.g. S = 0.958 between AK-1 and OK-1, Table 6). In many cases, the S values of populations from supposedly different subspecies equalled or exceeded values expected for local populations of the same subspecies (eg. S = 0.984 between MT-1 and MO-1, and S = 0.867 between CA-3 and ID-1 Table 6 and Fig. 1 and 2, p. 10 and 14).

The observed clustering of coyote populations relative to their allelic content is the result of fixed (or nearly so) differences at four loci. The most important of which is the SOD1 locus. All populations in each cluster share the same allele at that locus, whereas populations in different clusters each share an alternate allele. Since even a single genetic locus can be diagnostic with a high degree of confidence in identifying sibling species (Ayala and Powell 1972), it seems reasonable that a single locus could be useful in identifying different subspecies.

Three populations from California (CA-1, CA-2 and CA-3) form the most divergent group of coyotes observed. They were fixed for the same allele ("B") at SOD1, and at four other loci (GPD2, GPD3, G6PD and MDH1; Table 3, p. 20) were either fixed for an alternate allele or had a high frequency of an allele that was absent or rare in all other

populations. The CA-4 population, though similar to CA-1, 2 and 3 in sharing the "B" allele at SOD1, differs from these populations at several other loci. CA-4 clustered more closely with two populations, NE-1 and TX-2, of the south-central plains coyotes.

ME-1, the sole representative of New England coyotes in this study, is characterized by the "D" allele at SOD1. This population also shows relatively high similarity to the coyotes of the northern plains (MO-1, MI-1 and MT-1). The North-east plains coyotes form the largest cluster of genetically similar populations. This group, rooted at 0.960, is composed of five populations (MI-1, MO-1, MT-1, OK-1 and TX-1) representing three separate subspecies. Members of this group are more closely related to each other than are members of any other cluster, with a mean S value of 0.968 (range 0.945-0.984). The two remaining populations examined, AK-1 and ID-1, share the "C" allele at the SOD1 locus with the north-east plains cluster but, due to differences at other loci, branch from that cluster at an unresolved node at 0.945.

Only members of the subspecies C.l. ochropus, represented by CA-1 and CA-2, form a group consistent with current subspecific designations. However, CA-3, a representative of C.l. lestes, also is closely related to CA-1 and CA-2 with a mean similarity value of 0.935. The

last Californian population from the extreme southern portion of the state, (CA-4), is isolated from the other California populations by a division of the Coast Range mountains, which divides the San Joaquin Valley from the coastal region of San Diego county. Gene flow with the northern California populations, though likely, as can be seen by the sharing of the "B" SOD1 allele, is evidently reduced. CA-4 appears to have a higher degree of genetic similarity to the populations of the south-central plains coyotes. It is possible that these three populations may represent a widely distributed southern subspecies that extends from south Texas, through Mexico and into southern California. More data, especially from geographically intermediate populations are needed in order to test the validity of this hypothesis.

Coyotes from the north-east plains populations cluster very close together. From the data collected, it is not possible to separate any of these populations into one of the currently recognized subspecies (C.l. lestes, C.l. thamnos and C.l. frustror). The low degree of differentiation among these plains populations indicates little restriction of gene flow across large areas. There appears to be little or no genetic basis to the separation of these populations into different subspecies.

The New England coyote, C.l. variant has has been the subject of considerable debate for the last two decades. The arguments center around the origin of the animal, and the possibility of introgressive hybridization with the Ontario wolf, Canis lupus lycaon (Lawrence and Bossert 1969; Kolenosky 1971; Schmitz and Kolenosky 1985). It is generally agreed that coyotes in New England migrated out of Minnesota and Michigan into Ontario around the turn of the century, passed through southern Canada, and into New England by the mid-1950's (Silver and Silver 1969; Mengel 1971; Gipson 1978; Nowak 1979). Nowak (1979) classifies the New England coyote as a new subspecies, C.l. variant, whereas Hall (1981) includes it with C.l. thamnos (see Fig.1, p. 10). On the basis of the allozyme data presented in this study, and in spite of the presence of the unique allele "D" at SOD1, the New England coyote is quite similar to the north-east plains cluster with an average S of 0.942 (range 0.933-0.948).

Traditionally, the assignment of subspecies for coyotes was based on cranial measurements and pelage color. Of the 20 subspecies currently recognized, nine were described before 1900 and all others except C.l. hondurensis and C.l. variant were described prior to 1920 (Young and Jackson 1951; Hall 1981). In 1951, there was a revision of the nomenclature of Canis latrans, but it did

not attempt to quantify the available morphological data (Young and Jackson 1951). Since then, there has been no comprehensive analysis of the characters on which the current subspecies are based. More recent morphological studies have usually focused simply on assigning coyotes that are invading a particular region, to one of the current subspecies (eg. Gipson et al. 1974; Smith and Kennedy 1985).

The current taxonomy of subspecies in coyotes appears similar to that of the grey wolf (Canis lupus). At the present time, the grey wolf is divided into 24 subspecies (Hall 1981). To test the validity of the numerous grey wolf subspecies, Jolicoeur (1959) used mulitivariate statistics to analyze morphological characters in twelve populations representing six subspecies in Alaska and northern Canada. Jolicoeur (1959) was unable to identify more than two distinct clusters. One "cluster" was from Vancouver Island off the coast of British Columbia. The other cluster was from the mainland, and was composed of the remaining eleven populations. Though there was some separation of the mainland populations, he stated that there were too many recognized subspecies of grey wolf. Such a morphological study is lacking for coyotes, but it seems reasonable to assume that at least many of the current subspecies are probably not based on any quantifiable measure.

Genetic Variation Within Populations

In six populations examined (AK-1, CA-1, CA-4, ME-1, MI-1 and OK-1), at least one polymorphic locus differed significantly from Hardy-Weinberg expectations (Table 5, p. 24). In every case, the deviation involved a deficiency or absence of heterozygotes. There are several possible explanations that could account for this result. One possibility is disruptive selection where there may be selection against the heterozygotes. This is not a likely explanation because there was no consistent pattern as to which locus was out of equilibrium (i.e. no single locus diverged from Hardy-Weinberg equilibrium in more than one of the six populations). If disruptive selection were occurring, populations from similar habitats would be expected to have a deficiency of heterozygotes at the same locus. A second possibility is that inbreeding was occurring in the populations. The data indicate that this also is unlikely. In all populations, except CA-4, the number of loci out of equilibrium was less than 20% of all polymorphic loci scored. If inbreeding were the cause of the reductions of heterozygotes, it is expected that all polymorphic loci would be affected similarly. A more plausible interpretation is that the reduction of heterozygotes is the result of the Wahlund effect. This

phenomenon results in an apparent deficiency in the number of heterozygotes observed in a sample. The Wahlund effect occurs when several separate demes are collected for analysis and are assumed to represent a single panmictic population. The result is that there is an apparent decrease in the heterozygosity of the sample. The decrease equals twice the variance of the average allele frequency between the different demes (Futuyma 1986). In this study, samples were collected over large areas, often including animals from several counties (see Table 1, p. 13). This area is several orders of magnitude greater than the average coyote home range of ca. 21 km^2 (Springer 1982; Andelt 1985). As a result of the collection protocol, it seems probable that several different demes may have been included in some of the sample populations, and that the heterozygosities observed in those particular samples are less than estimated. Observation of microgeographic variation between populations would lend additional support to the Wahlund arguement. With the possible exception of California populations, detection of interpopulation variation is not possible with this data set due to the rather coarse-grain method of sampling. Because of their close proximity to each other, the California populations provide the best data from which to judge the Wahlund effect arguement. At locus AK1, CA-2 has a high percentage

of an allele that is at low frequency in CA-3 and occurs at a 50:50 ratio in CA-1. Other examples of local allele differences also can be seen in MDH1 and ODH2 (Table 3, p,20-21). Technical error also could be a possible reason for the observed deviations from Hardy- Weinberg expectation. This possibility seems unlikely because, (1) all the loci were scored by two different individuals and (2), several easily scored enzymes (eg. GOT1) were out of equilibrium in one or more populations and the likelihood of scoring error for these loci is extremely low, and (3) in no case did a locus deviate from Hardy-Weinberg expectations in more than one population.

A persistent generalization in the literature is that large mammals are genetically less variable than small ones (Wooten and Smith 1985). Selander and Kaufman (1973) proposed, that because large animals experience their environment in a more fine-grained manner, they are better insulated from environmental fluctuations than are small animals. This allows large mammals to have reduced genetic variability. Recently, numerous exceptions to the hypothesis that large mammals are genetically less variable have been reported (eg. Baccus et al. 1983; Werbitsky and Kilpatrick 1987). From these studies, it appears that the rarity of a particular species and, not its size may be the reason for that taxa having reduced genetic variability

More studies on large, common mammals and small rare mammals may help to clarify this situation.

In this study, the mean heterozygosity of Canis latrans was 0.034 ± 0.017. This value is very close to the mammalian average of 0.036 (Nevo 1978). The similarity of the coyote to the mammalian average is also apparent in the percent of loci polymorphic. The coyote's average was 14.93%, while the mean value recorded for mammals was 15.85% (Nevo 1978). The agreement of the data from the coyote and the Class average leads to the conclusion that this large mammal does not have reduced genetic variability. Data collected in this study provide an example of a large, common mammal which has genetic variability similar to that of other mammalian taxa. Low heterozygosity estimates for large mammals may be a fair generalization from the available literature (eg. Wooten and Smith 1985). However, many studies on large mammals have necessarily had small sample sizes or made use of captive populations (Wayne and O'Brien 1987; Fisher et al. 1976 Simonsen 1976) and it may be these logistic features have contributed to lower heterozygosity estimates. Ideally, to precisely estimate the heterozygosity of a species, not only should a large number of genetic loci be examined (Gorman and Renzi 1979), but samples should also be taken from different parts of the

range. It is important to note that different populations of coyote had substantially different levels of heterozygosity, depending on the population sampled (Table 4, p.22). Populations from California had the highest observed heterozygosity (X = 0.046), whereas the north-east and south-central plains coyotes were significantly lower (X= 0.028 and 0.011, respectively). The high heterozygosity values for the California coyotes may be the result of these animals living in larger, more stable populations, than the plains coyotes. Plains populations are apparently subject to heavy annual reductions of their numbers due to hunting and trapping for fur and pest control. These activities may so reduce the populations that there is a subsequent reduction in their genetic variability.

Conclusions

The major results of this study are twofold. First, my data call into question the validity of the current subspecific designation of coyotes based solely on morphological characters. A thorough taxonomic revision is clearly in order, and this revision should involve analysis of both morphological and genetic characters. When such a study is done, it will greatly add to our understanding of the phylogenetic history and biogeography of this group.

The second result of this study is to challenge the generalization that large mammals are, as a rule, low in genetic variability by presenting another exception to this notion. In this regard, I emphasize that for precise estimates of the variability of a species to be obtained, both a large number of loci, and sample populations from several different parts of the species range should be included.

LITERATURE CITED

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Allendorf, F.W., F.B. Christiansen, T. Dobson, W.F. Eanes, and D. Frydenberg. 1979. Electrophoretic variation in mammals I. the polar bear, Thalarctos maritimus. Hereditas 91: 12-22.

Amadon, D. 1949. The seventy-five percent rule for subspecies. Condor 51: 250-258.

Andelt, W.F. 1985. Behavioral ecology of coyotes in south Texas. Wildl. Monogr. 94: 1-45.

Andrews, R.D. and E.K. Boggess. 1978. Ecology of coyotes in Iowa. In Coyotes: Biology, Behavior, and Managment, ed. M. Bekoff. pp. 249-266. Academic Press, New York.

Avise, J. C. 1975. Systematic value of electrophoretic data. Syst. Zool. 23: 465-481.

Avise, J.C., M.H. Smith and R.K. Selander. 1974. Biochemical polymorphism and systematics in the genus Peromyscus. VI. The boylii species group. J. Mamm. 55: 751-763.

Ayala, F.J. 1975. Genetic differentiation during the speciation process. Evol. Biol. 8: 1-78.

Ayala, F.J. 1982. Population and Evolutionary Genetics: A Primer. The Benjamin/Cummings Publ. Co. CA.

Ayala, F.J. and J.R. Powell. 1972. Allozymes as diagnostic characters of sibling species of Drosophila. Proc. Natl. Acad. Sci. USA. 69: 1094-1096.

Baccus, R., N. Ryman, M.H. Smith, C. Rueterwall and D. Cameron. 1983. Genetic variability and differentiation of large grazing mammals. J. Mamm. 64: 109-120.

Bekoff, M. and M.C. Wells. 1981. Behavioral budgeting by wild coyotes: the influence of food resources and social organization. Anim. Behav. 29: 794-801.

Bekoff, M. and M.C. Wells. 1986. Social ecology and behavior of coyotes. Adv. Study Behavior 16: 251-338.

Berg, W.E. and R.A. Chesness. 1978. Ecology of coyotes in northern Minnesota. In Coyotes: Biology, Behavior and Managment, ed. M. Bekoff. pp. 229-247. Academic Press, New York.

Bowen, W.D. 1981. Variation in coyote social organization: the influence of prey size. Can. J. Zool. 59: 639-652.

Bowen, W.D. 1982. Home range and spatial organization of coyotes in Jasper National Park, Alberta. J. Wildl. Mgmt. 46: 201-216.

Bowen, W.D. 1984. Coyote. In The Encyclopedia of Mammals, ed. D. MacDonald. pp. 62-63. Facts on File Inc, New York.

Buth, D.G. 1984. The application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst. 15: 501-522.

Calhoun, S.W., I.F. Greenbaum and K.P. Fuxa. 1988. Biochemical and karyotypic variation in Peromyscus maniculatus from western North America. J. Mamm. 69: 34-45.

Caturano, S.L. and D.J. Harrison. 1981. The Coyote: a look at his life style. Maine Fish and Wildl. 23: 19-23.

Chesser, R.K. 1983. Genetic variability within and among populations of the black-tailed prairie dog. Evol. 37: 320-331.

Chesser, R.K., C. Reuterwall, and N. Ryman. 1982. Genetic differentiation of Scaninavin moose Alces alces over short geographic distances. Oikos 39: 125-130.

Clark, P., G.E. Ryan, and A.B. Czuppon. 1975. Biochemical genetic markers in the family Canidae. Aust. J. Zool. 23: 411-417.

Cole, S.R., P.R. Baverstock, and B. Green. 1977. Lack of genetic differentiation between domestic dogs and dingoes at a furthur 16 loci. AJEBAK 55: 229-232.

Darwin, C. 1859. The Origin of Species. John Murray, London. Modern Library, New York.

Endler, J.A. 1977. Geographic Variation, Speciation, and Clines. Monographs in population biology: 10. Princeton Univ. Press. Princeton, NJ.

Ferrell, R.E., D.C. Morizot, J. Horn, and C.J. Carley. 1980. Biochemical markers in a species endangered by introgression: the red wolf. Biochem. Genet. 18: 39-49.

Fichter, E., G. Schildman, and J. Sather. 1955. Some feeding patterns of coyotes in Nebraska. Ecol. Monogr. 259: 1-37.

Filippucci, M.G., G. Nascetti, E. Capanna and L. Bullini. 1987. Allozyme variation and systematics of European moles of the genus Talpa (Mammalia, Insectivora). J. Mamm. 67: 487-499.

Fisher, R.A., W. Putt, and E. Hackel. 1976. An investigation of the products of 53 gene loci in three species of wild Canidae: Canis lupus, Canis latrans, and Canis familiaris. Biochem. Genet. 14: 963-974.

Fitch, W.M. and E. Margoliash. 1967. Construction of phylogenetic trees. Science 155: 279- 284.

Futuyma, D.J. 1986. Evolutionary Biology. Second ed. Sinauer Associates, Inc. Sunderland, MA.

Gipson, P.S. 1978. Coyotes and related Canis in the southeastern United States with comment on Mexican and Central American Canis. In Coyotes: Biology, Behavior, and Managment, ed M. Bekoff. pp. 191-208. Academic Press, New York.

Gipson, P.S., J.A. Sealander and J.E. Dunn. 1974. The taxonomic status of wild Canis in Arkansas. Syst. Zool. 23: 1-11.

Gorman, G.C. and J.Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: the effects of sample size. Copeia 1979: 242-249.

Hall, E.R. 1981. The Mammals of North America. Second ed. John Wiley and Sons, New York.

Hamilton, M.J. 1984. Genetic variation in the Carnivora: I. Genetic variation in the raccoon, Procyon lotor. II. Genetic variation in the coyote, Canis latrans in Tennessee. Unpubl. M.S. thesis, Memphis State University. Memphis, Tennessee.

Hamilton, M.J., R.K. Chesser and T.L. Best. 1987. Genetic variation in the Texas kangaroo rat, Dipodomys elator Merriam. J. Mamm. 68: 775-781.

Harris, H. and D.A. Hopkinson. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. American Elsevier Publ. Co., Inc. New York.

Harrison, D.J. and J.R. Gilbert. 1985. Denning ecology and movements of coyotes in Maine during pup rearing. J. Mamm. 66: 712-719.

Hartl, G.B. and F. Csaikl. 1987. Genetic variability and differentiation in wild boars (Sus scrofa ferus L.): Comparison of isolated populations. J. Mamm. 68: 119-125.

Henshaw, R.E. 1982. Can the wolf be returned to New York? In Wolves of the World, ed. F. Harrington and P. Paquet. pp. 395-422. Noyles Publications, New Jersey.

Hillis, D.M. 1987. Molecular versus morphological approaches to systematics. Ann. Rev. Ecol. Syst. 18: 23-42.

Hilton, H. 1978. Systematics and ecology of the eastern coyote. In Coyotes: Biology, Behavior and Managment, ed. M. Bekoff. pp. 209-228. Academic Press, New York.

Johnson, W.E., R.K. Selander, M.H. Smith and Y.J. Kim. 1972. Biochemical genetics of sibling species of the cotton rat (Sigmodon). VII. Studies in Genet. Univ. of Texas Publ. 7213.

Jolicoeur, P. 1959. Mulitivariate geographical variation in the wolf Canis lupus L. Evolution 13(3): 283-299.

Kilpatrick, C.W. and E.G. Zimmerman. 1976. Biochemical variation and systematics of Peromyscus pectoralis. J. Mamm. 57: 506-522.

Kolenosky, G.B. 1971. Hybridization between wolf and coyote. J. Mamm. 52: 446-449.

Kurten, B. 1980. Pleistocene Mammals of North America. Columbia Univ. Press, New York.

Lawrence, B. and W.H. Bossert. 1969. The cranial evidence for hybridization in New England Canis. Breviora 330: 1-13.

Lawrence, B. and W.H. Bossert. 1975. Relationship of North American Canis shown by multiple character analysis of selected populations. In, The Wild Canids: Their Systematics, Behavioral Ecology and Evolution. ed. M.W. Fox. pp.73-86. Van Nostrand Reinhold Co. Toronto.

Mayr, E. 1940. Speciation phenomena in birds. Am. Nat. 74: 249-278.

Mayr, E. 1969. Principles of Systematic Zoology. McGraw-Hill Book Company, New York, NY.

McAlpine, P.J., T.B. Shows, R.L. Miller, and A.J. Pakstis. 1985. The 1985 catalog of mapped genes and report of nomenclature committee. Cytogenetics and Cell Genet. 40: 8-66.

McCullough, D.A. and R.K. Chesser. 1987. Genetic variation among populations of Mexican prairie dog. J. Mamm. 68: 555-560.

Mengel, R.M. 1971. A study of dog coyote hybridizations and implications concerning hybridizations in Canis. J. Mamm. 52: 316-336.

Messier, F. and C. Barrette. 1982. The social system of the coyote (Canis latrans) in a forested habitat. Can. J. Zool. 60: 1743-1753.

Moore, G.C. and J.S. Millar. 1984. a comparative study of colonizing and longer established eastern coyote populations. J. Wildl. Mgmt. 48: 691-699.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89: 583-590.

Nei, M. F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19: 153-170.

Nellis, C.H. abd L.B. Keith. 1976. Population dynamics of coyotes in central Alberta, 1964-1968. J. Wildl. Mgmt. 40: 389-399.

Nevo, E. 1978. Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. 13: 121-177.

Nowak, R.M. 1979. North american quarternary Canis. Monogr. Mus. Nat. Hist., University of Kansas, Lawreance. 6: 1-154.

Ortega, J. 1987. Coyote food habits in southeastern Arizona. The Southwest Nat. 32: 152-155.

Ozaga, J.J. and E.M. Harger. 1966. Winter activities and feeding habits of northern Michigan coyotes. J. Wildl. Mgt. 30: 809-818.

Ramsey, P.R., J.C. Avise, M.H. Smith and D.F. Urbston. 1979. Biochemical variation and genetic heterogeneity in South Carolina deer populations. J. Wildl Mgmt. 43: 136-142.

Rennert, P.D. and C.W. Kilpatrick. 1987. Biochemical systematics of Peromyscus boylii. II. Chromosomally variable populations from eastern and southern Mexico. J. Mamm. 68: 799-811.

Rogers. J.S. 1972. Measures of genetic similarity and genetic distance. Stud. in Genet. VII. Univ. of Texas Publ. 7213. 145-153.

Rohlf, F.J. and M.C. Wooten. 1988. Evaluation of the restricted maximum-likelihood method for estimating phylogenetic trees using simulated allele-frequency data. Evolution 42: 581-595.

Say, J. 1823. Account of an Expedition from Pittsburgh to the Rocky Mountains. Vol. 1. March of American Facsimile Series, Num. 65.

Schmitz, O.J. and G.B. Kolenosky. 1985. Wolves and coyotes in Ontario: morphological relationships and origins. Can. J. Zool. 63: 1130-1137.

Selander, R.K. and D.W. Kaufman. 1973. Geneic variability and stratagies of adaptation in animals. Proc. Nat. Acad. Sci. 70: 1875-1877.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson, and J.B, Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. variation in the old field mouse (Peromyscus polionotus). Studies in Genetics VI: 49-90. Univ. of Texas Publ. 7103.

Silver, H. and W.T. Silver. 1969. Growth and behavior of the coyote-like canid of northern New England with observations on canid hybrids. Wildl. Monogr. 17: 1-41.

Simon, C. and J. Archie. 1985. An empirical demonstration of the lability of heterozygosity estimates. Evolution 39: 7-18.

Simonsen, V. 1976. Electrophoretic studies on the blood proteins of domestic dogs and other Canidae. Hereditas 82: 7-18.

Simpson, G.G. 1961. Principles of Animal Taxonomy. Columbia University Press, New York, NY.

Smith, H.M. and F.N. White. 1956.A case for the triomen. Syst. Zool. 5: 183-192.

Smith, M.H., R.K. Selander and W.E. Johnson. 1973. Biochemical polymorphism and systematics in the genus Peromyscus. III. Variation in the Florida deer mouse (Peromyscus floridanus) a pleistocene relict. J. Mamm. 54(1): 1-13.

Smith, R.A. and M.L. Kennedy. 1985. Taxonomic status of the coyote in Tennessee. Unpubl. Results.

Sober, E. 1987. The Nature of Selection: Evolutionary Theory in Philosophical Focus. The MIT Press, Cambridge, MA.

Sokal, R.R. and P.H.A. Sneath. 1963. Numerical Taxonomy. W.H. Freeman and Co. San Francisco, CA.

Sokal, R.R. and F.J. Rohlf. 1981. Biometery. Second ed. W.H. Freeman and Co., New York.

Sudman, P.D., J.R. Choate and E.G. Zimmerman. 1987. Taxonomy of chromosomal races of Geomys bursarius lutescens Merriam. J. Mamm. 68: 526-543.

Springer, J.T. 1982. Movement patterns of coyotes in south central Washington. J. Wildl. Mgmt. 46: 191-200.

Swofford, D.L. and R.B. Selander. 1981. BIOSYS-1. Release 1.

Townsend, C.H. 1912. Mammals collected by the "Albatross" expidition in lower California in 1911, with descriptions of new species. Bull. Am. Mus. Nat. Hist. 30: 117-130.

Van Vuren, D. and S.E. Thompson. 1982. Opportunistic feeding by coyotes. Northwest Sci. 56: 131-135.

Wathen, W.G., G.F. McCracken, and M.R. Pelton. 1985. Genetic variation in black bears from the Great Smoky Mountains National Park. J. Mamm. 66: 564-567.

Wayne, R.K. and S.J. O'Brien. 1987. Allozyme divergence within the Canidae. Syst. Zool. 36: 339-355.

Werbitsky, D. and C.W. Kilpatrick. 1987. Genetic variation and genetic differentiation among allopatric populations of Megadontomys. J. Mamm. 68: 305-312.

Wilson, E.O. and W.L. Brown, Jr. 1953. The subspecies concept. Syst. Zool. 2: 97-111.

Woodruff, R.A. and B.L. Keller. 1982. Dispersal, daily activity and home range of coyotes in southeastern Idaho. Northwest Sci. 56: 199-207.

Wooten, M.C. and M.H. Smith. 1985. Large mammals are genetically less variable? Evolution 39: 210-212.

Young, S.P. and H.H.T. Jackson. 1951. The Clever Coyote. The Stackpole Co. Harrisburg, PA.

APPENDIX

GEL AND TRAY BUFFERS

1. POULIK pH 8.7. (Poulik)

   Gel: 76mM Tris-Hcl, 5mM Sodium Citrate.

   Tray: 300mM Boric Acid, 60mM Sodium Hydroxide, pH 8.2

   Run: 250V 3 hours.

2. TRIS BUFFER SYSTEM I: pH 6.3/6.7. (TC 6.7)

   Gel: 8mM Tris-HCl, 3mM Sodium Citrate, pH 6.7.

   Tray: 222mM Tris-HCl, 86mM Sodium Citrate, pH 6.3.

   Run: 150V 4 hours.

3. TRIS BUFFER SYSTEM II: pH 8.0. (TC 8.0)

   Gel: 687mM Tris-HCl, 157mM Sodium Citrate, pH 8.0.

   Tray: 1:30 dilution of gel buffer.

   Run: 125V 5 hours.

4. TRIS-MALEIC BUFFER SYSTEM, pH 7.4. (TM 7.4)

   Gel: 100mM Tris-HCl, 100mM Sodium Citrate, 11mM EDTA, 10mM Magnesium Chloride, pH 7.4.

   Tray: 1:10 dilution of gel buffer.

   Run: 100V 5 hours.

5. TRIS VERSENE BORATE BUFFER SYSTEM, pH 8.0 (TVB)

   Gel: 500mM Tris-HCl, 647mM Boric Acid, 18mM EDTA

   Tray: 1:10 dilution of gel buffer

   Run: 200V 4 hours.

VITA

Scott Hutcheson MacKenzie was born in Mt. Pleasant, New York on 22 January 1958. He lived in Harrison, New York until June of 1974, when his family moved to Silver Spring, Maryland. There he attended John F. Kennedy High School in Wheaton, Maryland, and received his high school diploma in 1977. In the fall of 1977, he began study at Montgomery College, with a major in history. In the fall of 1979 he entered The University of Maryland with a major in zoology, and in May 1982 received a Bachelor of Science degree in Zoology. Upon graduation from The University of Maryland, Mr. MacKenzie was employed at The National Cancer Institute, as a biologist. In the fall of 1985, he accepted a teaching assistantship at The University of Tennessee, Knoxville and began study toward a Master's degree. This degree was awarded in August 1988.

In June of 1979, he married Sheryl Lindinha. The next August, they had a daughter Loryn Christine MacKenzie. The author has accepted a graduate teaching assistantship at The University of Colorado, Boulder, for the fall of 1988, where he will pursue a Ph.D. degree.