Polyphyletic origins of extinct and extant domestic dogs

by

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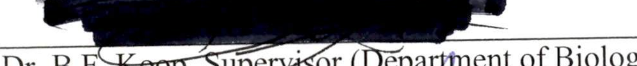

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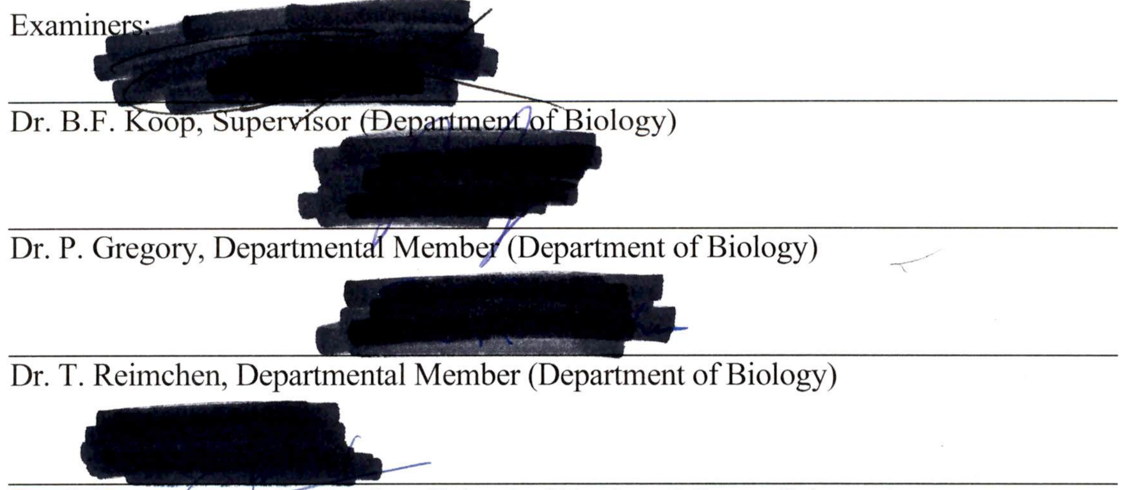
Abstract

A 718 bp region of the mitochondrial d–loop region in canids was used to infer phylogenetic trees for modern dog breeds, wolves, coyotes and a fox. A smaller 305 bp segment of the 5' hypervariable region within d–loop was obtained from museum skins and archeological bone of extinct Northwest Coast Native dogs (Tahltan Bear dog, Coast Salish "wool", "village" and historic dogs) and also included in the phylogenetic analyses. DNA sequence data generated by Okumura *et al.* (1996) and Vilà *et al.* (1997) were also analysed using phylogenetic methods. Eight groups were resolved: fox, coyote, wolf, wool dog and cohorts, North American indigenous hunting-type dog, modern dog, Japanese dogs D83611/D83637 and Norwegian elkhound. The data suggest that multiple domestication and introgression events have occurred between dogs and wolves. In light of this result, the validity of the dog and wolf species taxa is discussed. The distinct identity of two dog types kept by the Coast Salish of the Northwest Coast seems to be supported as well.



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"Common events in nature sometimes produce uncommon, puzzling designs. Even after we learn how the design was created, a sense of the inexplicable or a hint of mystery may linger on - making us aware that nature has more secrets than we can ever know."

-Freeman Patterson, *Portraits of Earth*

Introduction

Dobzhansky's (1973) statement that "Nothing in biology makes sense except in the light of evolution" is fundamental to our understanding of the natural world. To understand the evolutionary processes that regulate the biological world, one must employ a valid species concept. This is paramount because the species category is thought to be the only natural grouping within the manmade taxonomic hierarchy (Mayr 1982). It follows then that the accepted species concepts must reflect the natural processes that delimit species. Failure to consider on these processes could result in erroneous conclusions concerning the species under study.

Ideally, taxonomy should reflect phylogenetic relationships (Mayr 1982). Traditionally, these relationships have been inferred using morphological characters (Thorpe 1996), although the use of molecules for this purpose has increased substantially over the last two decades (Avise 1994). Some argue that molecules are superior to morphology to address phylogenetic questions, but others argue the opposing view (Eernisse & Kluge 1993). Often the results of these methodologies are congruent (Eernisse & Kluge 1993), but it is particularly interesting when the phylogenetic results of two methods are contradictory (see Thorpe 1996) because one is forced to determine the source of the disagreement. Occasionally this has resulted in recategorising a character (or suite of characters) as ancestral rather than informative (see Baker *et al.* 1995). Given the plasticity of morphological characters, one should not rely solely on morphometric data to delimit a species (typological species concept). Morphological differences or similarities may have arisen because of environmental factors rather than as a result of phylogenetic affiliation. Other factors, such as ecology, natural history, ethology or genetics, should be considered in order to arrive at valid conclusions regarding systematics and evolution of the species (Thorpe 1996).

Domestic species are difficult to deal with in terms of the present species concepts; however, studying the evolution of these animals and their phylogenetic relationships to their wild counterparts is useful in understanding evolutionary processes. One can study the effects of artificial selection on the morphology, behaviour and genetics of domestic animals and apply the theories to the natural world, as Darwin did to arrive at his theory of natural selection.

The original project was designed to determine if there is morphological and genetic evidence to support the existence of two distinct dog types reportedly kept by the Coast Salish and Makah Northwest Coast cultures of North America. Another objective of the project was to determine the relationship of these extinct dogs to extant domestic dog breeds, wolves and coyotes. A discriminant analysis of the archaeological remains of a large number of the "wool" dog and "village" dog types in Crockford's (1997) recent volume *Osteometry of Makah and Coast Salish Dogs* provides the morphological data pertaining to this project. Other recent works (Amoss 1993; Schulting 1994; Schwartz 1997) provide a substantial amount of historical information about these dogs. This work will therefore focus on the molecular analysis of the extinct dogs and their genetic relationships to modern dogs, wolves and coyotes.

1.1 Objectives

The history of the domestic dog (*Canis familiaris*) has captivated the minds of many scientists, historians and others for ages. Although modern researchers have revealed that the wolf (*C. lupus*) is most likely the ancestor of the dog (Wayne 1986a, b, c; Wayne & O'Brien 1987; Morey 1992; Clutton-Brock 1995), other questions remain unanswered. It is still unclear whether all dogs are a result of a single domestication event or if several events occurred (Morey 1992; Clutton-Brock 1995). The answers to other common questions such as why and how the domestication of the wolf occurred are more complex.

Genetic methods can be employed to investigate domestication issues and the occurrence of introgression, as well as to identify variation among dog breeds and other canid populations, (see Seal 1975; Wayne & O'Brien 1987; Wayne *et al.* 1989, 1994; Lehman *et al.* 1991, 1992; Tanabe *et al.* 1991; Wayne & Jenks 1991; Juneja & Shibata 1992; Tsuchida & Ikemoto 1992; Umenshi *et al.* 1993; Wayne 1993; Okumura *et al.* 1996; Vilà *et al.* 1997). Vrana *et al.* (unpublished data) studied genetic similarity among dog breeds and between dogs and wolves by comparing fragment sizes of mitochondrial DNA (mtDNA) cut with restriction enzymes. Their results indicated a high degree of similarity among canids, but the number of sites were insufficient to resolve several of the questions. The low resolving power of this and other mitochondrial characterisation methods in canid studies (Wayne *et al.* 1989; Juneja & Shibata 1992; Tsuchida & Ikemoto 1992; Wayne 1993) suggests that sequencing the d-loop would be the next logical step in attempting to answer questions relating to the dog and its relatives. It was anticipated that the d-loop of the mitochondrial genome would provide sufficient genetic variation to allow one to distinguish among canid species, as well as among dog breeds. It should also be possible to determine the occurrence of domestication and introgression events during the history of the dog, based on branching patterns within a phylogenetic tree. Previous studies have used sequence data from the mitochondrial genome to show that extensive introgression occurs among canid species in North America (Lehman *et al.* 1991; Wayne & Jenks 1991; Roy *et al.* 1996). With the genetic tools and analysis methods currently available, it was envisioned that some of the perplexing questions about dogs and their relationships could be answered.

The added dimension of being able to determine the history of extinct dogs is an intriguing part of this project. "Ancient DNA" techniques can be used to extract DNA sequence information from archaeological bones of three Coast Salish dog types and from museum skins of the Tahltan bear dog. One can use the DNA sequence information to

determine how these extinct indigenous British Columbian dogs were related to modern wolves, coyotes and dogs. These types of studies have caused much excitement within the fields of systematics and evolution because they have the potential to resolve the taxonomic placement of problematic endangered and extinct species while limiting the use of destructive or invasive sampling methods (see Higuchi *et al.* 1984, 1987; Johnson *et al.* 1985; Thomas *et al.* 1989; Wayne & Jenks 1991; DeSalle *et al.* 1992; Cooper *et al.* 1992; Kringer *et al.* 1997).

1.2 Natural History

The dog was the first animal to be domesticated; undisputed remains have been found in conjunction with humans as long ago as 14,000 BP (Morey 1992; Clutton-Brock 1995). Gidley's (1913) comparative study of fox, coyote, wolf and dog teeth provided some of the earliest scientific evidence that dogs and wolves are closely related, much more closely than either are to fox or coyote. The question still remained: was the wolf the ancestor of the domestic dog or did the two merely share a common ancestor (Allen 1920)? According to Olsen (1995), the domestic dog evolved from a wolf ancestor via the interference of man. There is no consensus on how the domestication of the dog occurred, but somehow humans and the ancestral dog entered into a mutually beneficial relationship (McLoughlin 1983; Clutton-Brock 1984; Morey 1994).

Dogs appear in the archaeological record in several locations nearly simultaneously throughout the world about 14,000 years ago, supporting the theory of multiple domestication events (Clutton-Brock 1984, 1995; Olsen 1985; Morey 1992). By 11,000 BP several regional varieties were present throughout the world, and by the first century BC all of the prototype "breeds" were present in Egypt (Clutton-Brock 1991). Based on presumed geographical origins, dog experts (Clutton-Brock 1984; Juneja & Shibata 1992) divide the old breeds into four stock groups (fig. 1.1), from which all other breeds were

derived. These four groups, with examples of each, are: 1) east Siberian spitz (samoyed, malamute), 2) west Siberian spitz (Norwegian and Swedish elkhound), 3) sighthounds (greyhound, whippet) and 4) flock guardian (great Pyrenees, maremma). Debate arises, though, in trying to determine from which wolf subspecies each stock group was derived. The stock breeds may have arisen from only the European grey wolf (*C. l. lupus*) or from many wolf subspecies (Morey 1994). For example, the Tibetan or Chinese wolf (*C. l. chanco*) may have been the ancestor of the flock guardian group, and the greyhounds may be descended from either the Asian or Indian wolf (*C. l. pallipes*) [Fiennes & Fiennes 1968; McLoughlin 1983] or from the Arab wolf (*C. l. arabs*) [McLoughlin 1983]. Some early workers suggested that other canid species were the progenitors of at least some breeds. Gülderstadt and Palles suggested the Indian jackal as the ancestor of European dogs (Allen 1920), and others proposed that the sighthounds also descended from the jackal (McLoughlin 1983). These hypotheses are based on phenotypic similarities (superficial resemblances) of sighthounds and jackals, but the likenesses are probably a result of morphological characters converging under similar selective pressures in the desert environment. The major morphological differences among dog breeds could be a result of several regional domestication events.

Determining the relationships among dog breeds using morphological characters has resulted in a weblike family tree rather than a typical tree (McLoughlin 1983). Many of the "newer" breeds (those that have existed for a few hundred years or less) were developed by crossing two or more ancient breeds, by crossing ancient breeds with newer breeds, or by crossing only newer breeds. The problem of determining breed relationships is compounded by the lack of written records for the development of most breeds; often one can only make suppositions with respect to the ancestry of some of the newer breeds. To further complicate matters, the hardiness of some breeds may have been enhanced via human directed back-crosses to wolves. Man's quest to improve breeds and to create new

ones has obscured the origins and history of many breeds.

1.3 Modern Dog Breeds

Unfortunately, there is very little refereed information available for most dog breeds; in lieu of scientifically verified knowledge, popular beliefs tend to be most widely represented in "dog fact" literature. Dogs are a part of human oral traditions and relatively little information pertaining to breed history has been recorded or substantiated. This is complicated by the historical context of the word "breed." As recently as the early 1800s, two dogs from the same litter would be referred to as different breeds if they had different coat colours (Glyn 1967). Breed standards as we know them today began to arise with the formation of the Birmingham Dog Show Society in 1860 (Glyn 1967). Presented here is an amalgamation of anecdotal information from numerous dog books (Browne 1974; Pugnetti 1980; Emert 1985; Palmer 1991; Jankowski 1994) regarding the contemporary breeds represented in this study. A few well investigated works also are presented in the following review (Glyn 1967; McLoughlin 1983; Dennis-Bryan & Clutton-Brock 1988; Juneja & Shibata 1992). Unless referenced, the majority of this information is unsubstantiated and only represents popular beliefs about dog breeds.

Northern spitz dogs. The northern or spitz breeds are presumed to have originated in the polar regions, and hence are sometimes referred to as polar dogs. They were and still are used for freighting, hunting, protection and companionship. The breeds are typically medium-sized, robust and powerful with a double coat, curled tail and erect ears. Their behaviours are similar to those of wolves in that they are pack-oriented and exhibit intelligence (McLoughlin 1983). According to popular belief, some of these breeds were occasionally back-bred to wolves to maintain their sturdiness in the harsh northern environment. The name husky reflects this back-breeding; a husky is defined as a mixture of northern breeds and often wolf (Dennis-Bryan & Clutton-Brock 1988). The purebred

northern dogs fall into two categories, the east Siberian spitzes and the west Siberian spitzes.

Among the many pure breeds included in the northern dog category are the Alaskan malamute, samoyed, Siberian husky, and akita inu, which are considered to have been derived from the east Siberian Laika dog populations (Juneja & Shibata 1992). Most sources presume that the Alaskan malamute descended from the Arctic wolf (*C. l. arctos)*, and that it is one of the few breeds that is still very close to its original function and form. These large dogs are used for hauling freight across the frozen tundra and to track large game by the Mahlemiut (an Alaskan Inuit tribe on the western shore of Alaska). Other remote northern tribes also kept work dogs. One such tribe was the Samoyedes, a group of hunters and fishers in Siberia. They used their dogs, samoyeds, to herd and guard reindeer, to hunt sable and walrus, to haul sleds and to act as watchdogs, helpers and household companions. Some sources suggest that the Samoyedes called their dogs Bjelkiers, meaning "white dogs that breed white" and kept the combings of the dogs to spin them into yarn for clothing. Many authors insist that the samoyed was bred pure (i.e. they were never crossed with wolves or any other dogs) for thousands of years before "discovery" by Arctic traders. According to Glyn (1967), however, the original breed was frequently crossed with other breeds and was not always white. The modern breed was founded shortly after the first white samoyed was imported to Britain in 1889. Another Siberian native breed, the Siberian husky was supposedly bred pure by the Chukchi people for at least 3,000 years before it was introduced to the North American Arctic in 1909. The Chukchi inhabited the basin of the Kolyma river at the foot of the Cherski Mountains in Northeast Siberia. Like the Samoyedes, the Chukchi were hunters of reindeer and used the purebred dogs to herd the animals, and to pull sleds. A third spitz dog of Asian origin is the akita inu. It is the national dog of Japan and is believed to be the oldest Japanese breed. The akita is the largest of the country's six spitz-type breeds. The breed is native to the

Honshu Island in the province of Akita and may have descended from the extinct Tengger dog of Java. Dogs very similar to the current akita breed are depicted in artwork dating back to 2,000 BC, and its written history goes back at least three hundred years. Like other dogs in the spitz group, the akita was once used for hunting, but as the Japanese lifestyle changed from that of hunter-gatherer to agriculturist, new uses were found for the breed. The strongest dogs were often bred for dog-fighting. Approximately one hundred years ago, the akita was crossed with other large, strong breeds (such as the German shepherd dog, Tosa fighting dog and English mastiffs) to augment its strength and height for more interesting pit-fighting. With the advent of other more efficient fighting breeds and the outlawing of the sport, the popularity of the akita dwindled. The breed was saved from extinction when, in 1919, the Japanese government declared it be preserved as a national monument. By 1931 the breed had been reestablished as the currently accepted akita inu; however, the breed went through another bottleneck during World War II and was again nearly lost. For a second time the breed was reestablished from the best remaining dogs. The malamute, samoyed, Siberian husky and akita are all considered stock breeds within the east Siberian spitz group.

The other northern stock group is thought to have descended from the west Siberian Laika populations of dogs and includes the Norwegian elkhound, Finnish spitz and Swedish elkhound (Juneja & Shibata 1992). Like most northern breeds, the compact Norwegian elkhound was a working dog, used for pulling sleds, herding and protection as well as for hunting. In 1913 the first registered "moosedog" was imported to North America (Wallo 1957), where the breed still remains relatively rare. The Norwegian elkhound is one of four Scandinavian spitz breeds: the others are the Swedish elkhound (or jämthund), the rare black elkhound and the Norwegian buhund (Glyn 1967). Glyn suggests that Scandinavian villages maintained different variations on the elkhound, and that it is these regional types which are now considered different breeds. Some suggest

that the elkhounds originated 6-10,000 years ago (BP) because of their similarity to archaeological remains found in Scandinavia. Perhaps these accounts refer to the Varanger dog, which is found in Norway as far back as 7,000 BP or to the 5,000 year old elkhound-like dog remains found throughout the Baltic region (Glyn 1967). However, no known morphometric work has compared these archaeological dogs to contemporary elkhounds, nor to any of the other breeds within the west Siberian spitz group.

Sighthounds. The sighthounds (also referred to as greyhounds or gazehounds) are thought to be a very old group of breeds, based on interpretations of ancient art in the Middle East. Sighthounds are adapted to hunting on the open plains and deserts of their native habitat; they rely on their sight to spot quickly moving prey and on their great speed to overtake and bring down their target. Their keen eyesight and sprinting ability were enhanced by selective breeding to improve on their natural hunting skills (McLoughlin 1983). The sighthounds are characterized by a slight build, long slender legs, a narrow head and a long tail (Dennis-Bryan & Clutton-Brock 1988). Breeds included in this group are the greyhound, saluki, whippet, ibizi hound, sloughi, afghan, Scottish deerhound, borzoi (or Russian deerhound), the Italian, Australian and English greyhounds. In Europe, the Celts and Russians purportedly developed the deerhounds and wolfhounds, while the breeding of sighthounds with northern dogs probably produced the collies and similar dogs (McLoughlin 1983).

Several new breeds fall into the sighthound group but only a few breeds are considered to be very old members of the group. Among the oldest sighthound breeds are the greyhound and saluki. The greyhound type of dog is 4,000 to 7,000 years old, descended from the pharaoh hounds of Egypt. The breed was later dispersed throughout Eurasia via traders, approximately 2,000 years ago. However the currently accepted breed standards describe the greyhound that was more recently developed in Britain (Dennis-Bryan & Clutton-Brock 1988). Like all sighthounds, the greyhound is a hunter,

used to hunt deer, wild boar and rabbit. It is also used as a racing dog, reaching speeds of 40 mph or more. The saluki also was bred as a hunter and used to hunt antelope, gazelle and hare in the eastern deserts. It is believed to have been developed by Arab tribesmen and to have been named for the ancient Arab city of Salug. It is one of the oldest sighthounds and is considered to be one of the world's oldest recognized breeds (McLoughlin 1983; Dennis-Bryan & Clutton-Brock 1988; Clutton-Brock 1991). By 7,000 BP, paintings of saluki-type dogs appeared in Assyrian temples, Egyptian tombs and Sumerian buildings. The saluki was exported from Persia to Britain in 1840 and finally gained popularity in the 1890s, causing more salukis to be imported and bred (Glyn 1967). The newest breed of sighthound, the whippet, was developed in Britain late in the nineteenth century. This was accomplished by crossing three breeds, greyhound, Italian greyhound (a miniature greyhound) and terrier. The greyhounds from which they were bred are supposed to have been imported to Britain during the time of the Roman invasion. The whippet is a very new breed of sighthound, unlike the very old greyhounds and salukis.

Flock guardian dogs. The flock guardian breeds descended from the ancient Tibetan mastiffs (Clutton-Brock 1984), which are thought to have originated at least 5,000 years ago in Tibet or northern India (McLoughlin 1983). Ancient Assyrian art depicting Tibetan mastiff-like dogs dates to 3,000 BP (McLoughlin 1983; Dennis-Bryan & Clutton-Brock 1988), and indications of similar dogs in ancient Greece and Rome also exist (Dennis-Bryan & Clutton-Brock 1988). It is normally accepted that the ancient Tibetan mastiffs were crossed with northern dogs to produce some of the largest and most massive dog breeds, such as the St. Bernard and the great Pyrenees. The great Pyrenees is thought to have originated in the Basque region of the Pyrenees Mountains, between France and Spain (Dennis-Bryan & Clutton-Brock 1988). It was originally used to guard flocks of sheep against wolves and bears in the mountains of the Ariège and is probably a

very old breed (Dennis-Bryan & Clutton-Brock 1988). They were originally exported to Britain in 1911 and were reintroduced to that island in 1933 when they became popular in Europe. The maremma sheepdog has always been a popular breed with shepherds. In the 1950s, it was established as a single breed from two very similar mountain breeds (the maremmano and the abruzzese). The two breeds differed only slightly in body size and coat length, and both were probably introduced to Italy by the Asian Magyars. These aggressive dogs were apparently among the first breeds to guard domestic sheep. Most of the flock guardian breeds are aggressive and/or protective, and thus have the predetermined temperament for guard duty. Breeds that seem to be unlikely descendants of the naturally aggressive mastiffs probably include retrievers, hounds, spaniels and poodles (McLoughlin 1983). These new breeds are much less aggressive than the old flock guardian breeds which gave rise to them.

Unknown origins. Although the shar-pei (once the rarest dog in the world) likely arose in Asia, its ancestral roots are uncertain. Likenesses of the shar-pei date to the time of the Han Dynasty (206 BC To 220 AD) exist, but they contribute little to the debate over the ancestry of the breed. Some people believe that the shar-pei is closely related to the chow chow because they both originated in China and have purple tongues, but further evidence of shared ancestry is sparse. Others concede that the breed may have originated in Tibet or northern China two thousand years ago but they insist that the ancestry of the shar-pei is unknown.

1.4 Ancient "Breeds"

Dogs also existed in the New World. The oldest domestic dog remains were found in Danger Cave, Utah and date to 9,000 - 10,000 BP (Morey 1992). Dogs were the only domestic animal to be found on the North American continent before the arrival of Europeans (Allen 1920; Morey 1992). European explorers of the fifteenth century

encountered seventeen distinct types of domestic dogs during their travels throughout the New World (Allen 1920; Haag 1948). Olsen (1985) suggests that all of these dog types, unlike American Kennel Club registered breeds, were freely breeding mongrels. Most of the New World dogs were found on the plains and in the Arctic (Allen 1920; Clutton-Brock 1991), but two varieties described by Allen (1920) also were found along the southern coast of British Columbia.

Archaeological data and ethnographic information support the theory that the Coast Salish people, of coastal southern British Columbia, Washington and northern Oregon (fig. 1.2), kept two distinct types of dogs. Early European explorers (e.g. Vancouver, Fraser, Lewis & Clark) and ethnographers (e.g. Boaz, Teit, Newcombe) of the northwest coast described two morphologically distinct types of domestic dogs possessed by the Coast Salish: the "village" dog and the smaller "wool" dog (Schulting 1994; Crockford 1997a). The village dog (fig. 1.3) was described as a coyote–like tan and white cur which was occasionally used for hunting. There is not as much detailed information about this dog as there is about the wool dog (fig. 1.4), a small to medium-sized Pomeranian-like dog, usually white or light in colour. Perhaps the village dog was overlooked because the wool dog was at the centre of a unique situation among North American indigenous cultures. Nowhere else in prehistoric North America had European explorers encountered a culture that utilised animal husbandry methods to obtain materials for textiles. The early explorers reported that wool dogs were kept on islands away from the main settlements to inhibit them from breeding with the village dogs, thus keeping the wool-producing line pure (Howay 1918; Schulting 1994; Crockford 1997a). The thick wool-like hair of the wool dog was shorn with a knife and used in the weaving of blankets and possibly in some clothing (Orchard 1926; Suttles 1990; Schulting 1994). The blankets were important economic and status symbols in the Coast Salish culture (Amoss 1993; Schulting 1994). They were a source of wealth and many were given away by the most affluent families at

potlatch ceremonies to establish social stature (Amoss 1993; Schulting 1994). However, once cheap European blankets and materials were available via trade with the Hudson Bay Company, the labour-intensive dog-hair blankets became passé (Suttles 1990). There was no longer an economic need to keep the two dog "breeds" separate; therefore, the wool dog became extinct as a distinct breed about a hundred years ago (Suttles 1990; Crockford 1997a). It is unknown whether the dogs simply died out or whether the wool and village dogs interbred with each other and with European dogs so that the Coast Salish dogs were no longer recognizable as two distinct breeds.

Bone samples collected from post-contact archaeological sites are referred to as historic dogs in this study, because the two Coast Salish dog types may have been allowed to interbreed after European contact. The size of the historic dog suggests that this dog may be a hybrid of a female village dog and male European breed (Crockford 1997a). Characteristics of the burial suggest that the historic dog was a valued First Nations hunting companion (Crockford pers. comm. 1997).

The people of the Tahltan nation also kept hunting dogs, the Tahltan bear dog (fig. 1.5). The Tahltan people traditionally occupied the Stikine plateau (see fig. 1.1) between the Coastal and Cassiar mountain ranges of northern British Columbia and southern Yukon (Albright 1984). Before the introduction of guns, the dogs often were carried to the hunting ground, then released to chase and corner grizzly bears, while the hunters followed with bows and arrows. The small size of these dogs (15-30 pounds and 14-16 inches at the shoulder) was conducive to agility and quickness. They easily darted around the hunted bear causing it to become aggravated and confused (Crisp 1956; Browne 1974; Albright 1983; Palmer 1991; Jankowski 1994). The bear dogs were apparently very affectionate and good with children, which is the only reason they were kept after guns became available to Tahltan hunters. The breed was registered with the Canadian Kennel Club (CKC) in the 1950s, but has since become extinct. The last registered Tahltan bear

dog died in the early 1990s, about a century after the breed was abandoned as a hunting companion.

1.5 Application of Molecular Data

One can use molecular data, especially ancient DNA data, to determine the ancestral and geographical origins of the Tahltan and Coast Salish dogs and to verify or refute the reports describing to the Coast Salish wool dog as a distinct breed from the village dog (see also Allen *et al*. 1996). One also could potentially resolve the debate as to whether the ancient dogs originated in Eurasia and crossed the Bering Strait land bridge with the First Nations peoples or whether the dogs were domesticated from local North American wolf populations. DNA is one of the favoured molecular markers utilised to study evolutionary and historical questions because of its hereditary properties and its nearly universal code in all forms of life. One can characterise, align and phylogenetically analyse homologous DNA sequences from a diverse array of organisms. One can also use ancient DNA techniques to extract information from extinct species. These methods can address many of the questions posed in this study.

A useful molecular marker for the study of evolution, systematics, population genetics and biogeography is the mitochondrial genome. This molecule (fig. 1.6) has been well studied and characterised in animals over the past two decades (Moritz *et al.* 1987) and is thought to evolve five to ten times faster than the nuclear genome (Brown, George & Wilson 1979; Brown *et al.* 1982; Wayne & Jenks 1991; Villablanca 1994). The small circular genome contains a non-coding control region, several transfer RNAs, ribosomal RNAs and genes that encode enzymes involved in the electron transport chain and ATP synthesis (Saccone *et al.* 1987). The genome has become a standard resource in many fields since it: (1) evolves rapidly, (2) lacks introns and pseudogenes, (3) is highly conserved in structure and arrangement of genes in mammals, (4) has a maternal mode of

inheritance, (5) is not subject to recombination events, (6) is present in high copy number within the cell (1,000–10,000 copies) and (7) replicates and divides independently of the nucleus (Hutchison *et al*. 1974; Giles *et al*. 1980; Anderson *et al.* 1981, 1982; Bibb *et al.* 1981; Watanabe *et al*. 1985; Moritz, Dowling & Brown 1987; Saccone *et al.* 1987; Wolstenholme 1992; Gradaleta 1989; DeSalle, Williams & George 1993). For these reasons and because it has been so well characterised in the recent literature, the mitochondrial genome has become a popular molecular marker in many fields of biology.

Different regions within the mitochondrial genome can be used to address questions pertaining to either distantly related groups (e.g. genera, families or classes) or to closely related groups (e.g. populations, species) because each mitochondrial gene evolves at a different rate (Villablanca 1994). The mitochondrial region chosen must be appropriate for the taxa to be studied, the type of data required and the type of study being pursued. Mitochondrial genes that encode proteins which are universally critical in function (e.g. cytochrome oxidases) evolve much more slowly than genes which are not as crucial across taxa. Generally, a protein or DNA molecule that has a critical function will acquire few changes, thus avoiding loss of function and decreased fitness of the organism (Kimura 1987; Villablanca 1994). These critical genes are useful for determining relationships between distantly related groups. Other more rapidly evolving regions are used to study relationships between and among lower taxonomic categories. The more rapidly a molecule evolves, the lower the taxa that can be studied with that particular molecule (Villablanca 1994). For example, because it is highly susceptible to insertions and deletions, the d-loop is the most variable region in the animal mitochondrial genome (Brown *et al.* 1986). This characteristic makes it suitable for differentiating between populations of a species (Thomas *et al*. 1990). Since it does evolve so rapidly, the d-loop becomes saturated and cannot be relied upon to supply phylogenetic information above the species level. The saturation point of a DNA sequence is reached when the real number of

nucleotide changes is masked by multiple changes at base sites (DeSalle, Williams & George 1993; Villablanca 1994). Cytochrome *b* evolves more slowly and is usually applied to interspecific studies, although it can be successfully used below the species level for some animals (Villablanca 1994). Since the regions of the mitochondrial genome evolve so unevenly, it is imperative that the researcher select a gene that will contribute reliable data to the investigation (Janczewski *et al.* 1992).

The d-loop was chosen for the present study because it evolves so rapidly. Another asset of the d-loop is that it is part of the mitochondrial genome and, therefore, is present in high copy number within the cell. Because the d-loop evolves so rapidly, it was anticipated that there would be sufficient variation to discern among closely related dog breeds and between wolf and dog. Also, because the mitochondrial genome is present in such large quantities within the cell, successful extraction is much more likely than for a single copy nuclear gene (Hagelberg & Clegg 1991; Hagelberg 1994). This is a particularly significant attribute of mitochondrial DNA for projects which include ancient DNA analysis, because DNA in ancient tissues is degraded and difficult to extract (Pääbo 1989). Mitochondrial DNA is also more likely than nuclear DNA to survive autolytic degradation and other damage to nucleic acids that occurs after the death of an organism (Pääbo 1991). These are significant reasons to use the mitochondrial genome and the d-loop in conjunction with ancient DNA techniques to determine the phylogenetic relationships among dog breeds, the occurrence of domestication events, the occurrence of introgression between dogs and wolves and the phylogenetic affinities of the extinct Coast Salish dogs and the Tahltan bear dog.

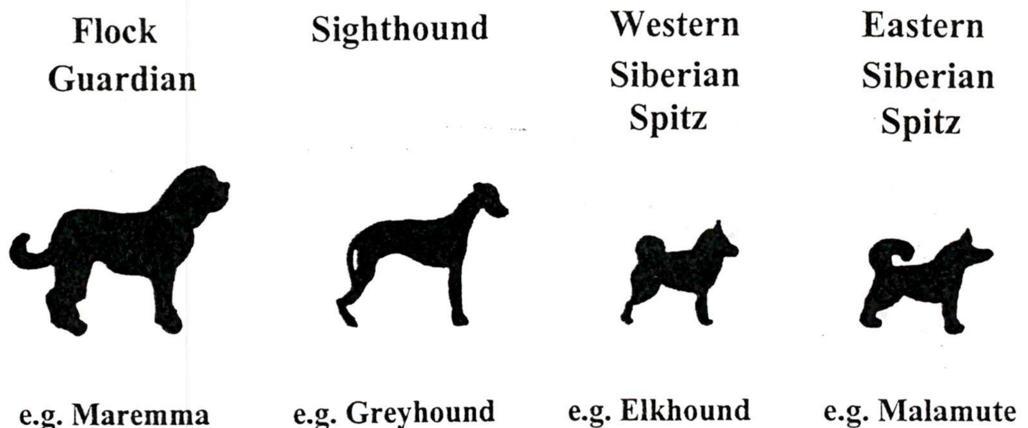


Fig. 1.1 The four major stock breed groups of dogs, from which all other breeds were developed (Clutton-Brock 1984; Juneja & Shibata 1992).

Fig. 1.2 Coast Salish and Tahltan territories. The map of British Columbia and northern Washington shows the traditional territories of the Coast Salish and Tahltan First Nations.

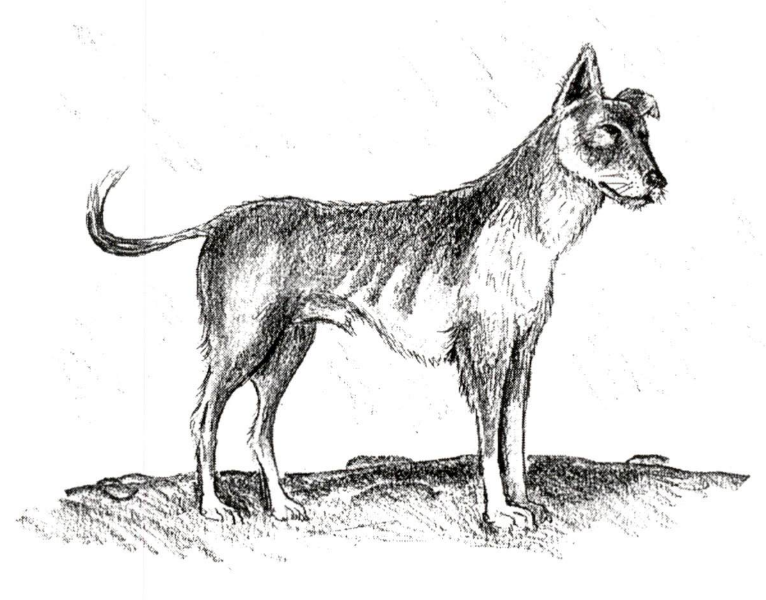


Fig. 1.3 The Coast Salish village dog. The reconstructive drawing was done by Cam Pye, a forensics artist in Vancouver, B.C.

Fig. 1.4 The Coast Salish wool dog. The reconstructive drawing was done by Cam Pye, a forensics artist in Vancouver, B.C.

Fig. 1.5 A Tahltan bear dog. "Iskut" was one of the last known Tahltan bear dogs; he died in 1982. Photo courtesy of Mrs. W. Acheson of Atlin, B.C.

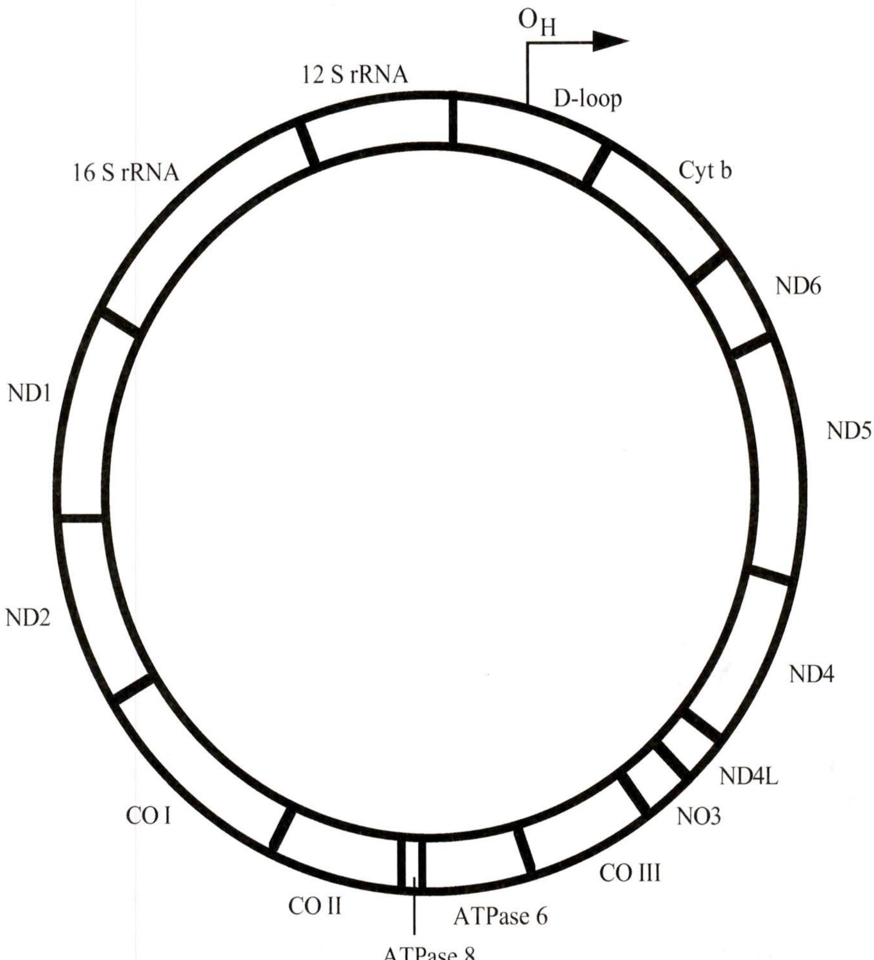


Fig. 1.6 Map of the mitochondrial genome in mammals. This simplified map shows the structure and arrangement of genes within the mammalian mitochondrial genome. O_H indicates the origin of replication of the H strand and the arrow indicates the direction of replication.

Materials and Methods

2.1 Samples

Samples were collected from twenty-nine extant dogs, five archaeological and museum dogs, thirteen wolves, three coyotes and a fox. Dog blood samples (table 2.1) were taken by local veterinarians from eleven modern dog breeds which are thought to have relatively unspoiled histories: great Pyrenees (n=2), malamute (n=4), Siberian husky (n=3), maremma (n=4), Norwegian elkhound (n=2), akita (n=2), shar-pei (n=1), samoyed (n=2), saluki (n=1), whippet (n=5) and greyhound (n=3). Blood samples also were collected from Arctic wolf (n=2), and liver and kidney tissue was harvested from one Vancouver Island wolf (individual 3). Muscle tissue was used for the rest of the wild canids: a fox, three coyotes, six Yukon wolves, two European wolves and two other Vancouver Island wolves (table 2.2). Ancient Coast Salish dog samples, wool dog (n=1), village dog (n=1) and historic dog (n=1), were powdered archaeological bone; the Tahltan bear dog samples (n=2) were museum skin clippings (table 2.3). Archaeological bluefin tuna (*Thunnus thynnus*) bones were used as a contamination control for drilling and extraction methods. All samples were stored at -70°C. Control region sequences from forty dogs (accession numbers U03575, Slade *et al.* 1994; D83599-D83638, Okumura *et al.* 1996; X97343, Rothuizen *et al.* 1996) and one fox (accession number D83639, Okumura *et al.* 1996) were obtained from the GenBank database and also included in the phylogenetic analyses.

2.2 Contemporary DNA Methodology

DNA extraction methods. Two methods, Chelex and DTAB/CTAB, were used to extract DNA from contemporary canid tissues (primarily blood and muscle). The Chelex method (Walsh *et al.* 1991) was used to extract DNA from blood samples (U.

Table 2.1. Dog breed samples used in this study. All breeds were dogs were from Victoria, B.C., and samples were collected by Dr. Layne Bixby of the Quadra Animal Clinic in Victoria.

SAMPLE	STOCK GROUP	NAME	OWNER
Akita 1	Western Siberian	Yuma	Vandevelde
Greyhound 2	Sighthound	Aurora	Dixon
Greyhound 3	Sighthound	Mistral	Dixon
Alaskan Malamute 4	Western Siberian	Ginger	Betanzo
Norwegian Elkhound 5	Eastern Siberian	Cody	Torgness
Saluki 6	Sighthound	Ryder	Doyle
Samoyed 7	Western Siberian	Leela	Chadwick
Samoyed 8	Western Siberian	Loki	Crossman
Siberian Husky 9	Western Siberian	Nico	Sitter
Siberian Husky 10	Western Siberian	Reese	Mokosak
Siberian Husky 11	Western Siberian	Simo	Kucheran
Whippet 13	Sighthound	Ceasar	Lucas
Whippet 14	Sighthound	Music	Lucas
Whippet 15	Sighthound	Polu	Fontaine
Whippet 16	Sighthound	Shadow	Dixon
Great Pyrenees 17	Flock Guardian	Busy Lizzie Gator	Armstrong
Whippet 18	Sighthound	Zoey	Greenhalgh
Norwegian Elkhound 19	Eastern Siberian	Misty	Walker
Alaskan Malamute 20	Western Siberian	Punuk	Crockford
Alaskan Malamute 22	Western Siberian	Zaur	Beck
Akita 23	Northern Spitz	Mitsu	Morton
Alaskan Malamute 24	Western Siberian	Nootka	Peckenpaugh
Shar Pei 25	Undetermined	Lady	Pellitier
Greyhound 26	Sighthound	Ptolemy	McCloy
Great Pyrenees 27	Flock Guardian	Meringue	Porter
Maremma 28	Flock Guardian	Shirley	Dewar Greene
Maremma 31	Flock Guardian	Joe	Dewar Greene
Maremma 32	Flock Guardian	Sumas	Dewar Greene
Maremma 33	Flock Guardian	Ben	Dewar Greene

Table 2.2 Contemporary wild canid samples and locations. Samples were donated by §Mary Gamberg, Watson Lake, Yukon, *Philip Merchant, Yukon Fish and Wildlife; †William A. Rapley, Metro Toronto Zoo; ±Drs. Coates and ¥C. Helen Schwantje, B.C. Wildlife, Victoria; and ‡Dr. D. Heinrich, Christian-Albrechts-Universität zu Kiel, Institut für Haustierkunde.

SAMPLE	TISSUE	LOCATION
Yukon Wolf 1*	muscle	Kusawa Lake
Yukon Wolf 2*	muscle	Whitehorse area
Yukon Wolf 4§	muscle	Watson Lake
Yukon Wolf 5*	muscle	Whitehorse area
Yukon Wolf 6*	muscle	Whitehorse area
Yukon Wolf 22*	muscle	Onion Lake/Nisling Lake
Vancouver Island Wolf 1¥	muscle	Vancouver Island
Vancouver Island Wolf 2¥	muscle	Vancouver Island
Vancouver Island Wolf 3¥±	liver & kidney	Vancouver Island
European Wolf 1‡	muscle	Germany (captive)
European Wolf 2‡	muscle	Germany (captive)
Arctic Wolf 1†	blood	Metro Toronto Zoo
Arctic Wolf 2†	blood	Metro Toronto Zoo
Red Fox§	muscle	Watson Lake, Yukon
Coyote 14*	muscle	Haines Junction, Yukon
Coyote 15*	muscle	Haines Junction, Yukon
Coyote 16*	muscle	Haines Junction, Yukon

Table 2.3 Ages and locations of ancient samples used in this study. Sample material was donated by the *Royal British Columbia Museum and †S. Crockford. Usable DNA could not be extracted from the samples below the double line. Ribs from an intact burial of specimen SM89:0400 were sampled for wool dog 64, from the 1989 salvage excavation site DgRr2, the St. Mungo Cannery site. An isolated femur of specimen WH88:1081 were sampled for village dog 74, from the 1988 salvage site DgRs30, the Beach Grove golf course on the Fraser Delta. Ribs, radius and ulna from an intact burial were sampled from Historic dog 41/42, obtained from the 1992 salvage excavation site DgRr1 on the Fraser Delta (Crockford 1997a). The tuna samples are also referenced in Crockford (1997b).

SAMPLE	TISSUE	AGE	LOCATION
Tahltan 2892*	museum skin	1938 AD	Liard River, BC
Tahltan 4758*	museum skin	1940 AD	Liard River, BC
Wool Dog 64†	archaeological bone	3000-4000 BP	Vancouver, BC
Village Dog 74†	archaeological bone	1400-2400 BP	Beach Grove, BC
Historic Dog 41/42†	archaeological bone	ca. 1800 AD	Crescent Beach, BC
Tahltan 5027*	museum skin	1944 AD	British Columbia
Wool Dog 17†	archaeological bone	1400 BP -	Little Qualicum R, BC
Wool Dog 65†	archaeological bone	ca. 1400 BP	Ozette, WA
Wool Dog 68†	archaeological bone	1400 BP -	Crescent Beach, BC
Village Dog 19†	archaeological bone	1400-2400 BP	Beach Grove, BC
Village Dog 72†	archaeological bone	ca. 500 BP	Ozette, WA
Village Dog 73†	archaeological bone	1400-2400 BP	Beach Grove, BC
Village Dog 74†	archaeological bone	1400-2400 BP	Beach Grove, BC
Prehistoric Dog 30†	archaeological bone	3000-4000 BP	Vancouver, BC
Prehistoric Dog 31†	archaeological bone	3000-4000 BP	Vancouver, BC
VI Wolf 53†	archaeological bone	1400-2400 BP	Ships Point, BC
VI Wolf 56†	archaeological bone	1400-2400 BP	Ships Point, BC
Tuna 34†	archaeological bone	730-1310 AD	Barkley Sound, BC
Tuna 35†	archaeological bone	730-1310 AD	Barkley Sound, BC

Rink, pers. comm. 1994). Three to 6 μL of whole blood were incubated 15 to 30 minutes at room temperature in 1 mL ddH_2O. A 5% Chelex solution was added to 20-30 μL supernatant to a final volume of 200 μL, incubated 15 to 30 minutes at 56°C, and then boiled for 8 minutes. This extraction method, when used in conjunction with PCR amplification of the canid d-loop, resulted in inconsistent banding patterns. The PCR products were often of bad quality and in many cases double bands (between 1,000 and 1,600 base pairs) resulted. A second purification round of PCR was attempted on isolated 1,100 bp PCR products, but the resulting DNA remained very difficult to sequence (U. Rink & B. Koop, pers. comm. 1994). The DTAB/CTAB DNA extraction method (Gustincich *et al.* 1991) was more successful for obtaining the proper PCR product. For this method, thawed tissue (250 μL aliquot of blood, or similar amount of other chopped tissue) was incubated in 2 volumes of lysis buffer (8% dodecyltrimethylammonium bromide [DTAB, Sigma], 1.5 M NaCl, 100 mM Tris-HCl, pH 8.6, 50 mM ethylenediamine tetraacetic acid [EDTA], pH 8) for 5 minutes at 68°C, and the resulting solution deproteinized with one volume of chloroform. One volume of ddH_2O was added to the aqueous layer, and the DNA precipitated with CTAB solution (0.3% hexadecyltrimethylammonium bromide [CTAB, Sigma] and 20 mM NaCl). Detergents were exchanged by dissolving the pellet in 250 μL 1.2 M NaCl, and the DNA recovered via ethanol-precipitation (Sambrock *et al.* 1989). DNA pellets were washed with 70% ethanol, dried and redissolved in 100 μL TE (Tris-EDTA), pH 7.5 (10 mM Tris-HCl, pH 7.5, 1 mM EDTA), aided by heating to 55°C. DNA extracts were visualised on 0.7% agarose (Dalton)/1X TAE (Tris-acetate-EDTA) gels and chemically amplified using the polymerize chain reaction (PCR). Amplification was much more successful from the DTAB/CTAB extracts than from the Chelex extracts, even though others have reported good success with the Chelex method (Walsh *et al.* 1991).

Amplification of DNA extracts. PCR reactions to amplify the extracted DNA were carried out in a 50 μL solution containing 1X PCR buffer (500 mM KCl, 100 mM $MgCl_2$ and 15 mM Tris-HCl pH 8.4), 200 μM each dNTP (2'-deoxynucleoside 5'-triphosphate, Pharmacia), 0.5 μM each primer, 0.1 units *Taq* DNA polymerase and 10.0 μL DNA template (regardless of concentration). Reactions were performed in either the Perkin Elmer 9600 or the PTC-200 DNA Engine (MJ Research). The PCR programme consisted of 5 minutes of melting at 94°C, followed by 30 cycles of denaturation (45 seconds at 94°C), annealing (45 seconds at 50°C) and extension (7 minutes at 72°C). Upon completion of cycling, the amplified DNA was elongated for an additional 7 minutes at 72°C. The primers used to amplify the 1.2 kb d-loop region were H15980 5'-GCTGG TACCACCATCAGCACCCAAAGCT-3' and L650 5'-RYTGGTACCAAGGCTRGGAC CAAACCT-3' (Dalton). The primers designed for canid mtDNA were based on previously published primers for the d-loop in several animals (Palumbi *et al.* 1991).

M13 cloning. The M13 cloning vector was cut with Kpn I by digesting 2 μg of M13mp18 vector DNA (Pharmacia) in a 103 μL reaction containing 1X concentration of OPA PLUS buffer and 60 units of Kpn I for 2 hours at 37°C. Completely digested vector was dephosphorylated with 3 units calf-intestine alkaline phosphatase (Boehringer Mannheim GmbH) for 20 minutes at 37°C, 20 minutes at 50°C and 30 minutes at 80°C. The vector preparation was extracted with 1 volume tris-equilibrated phenol (GibcoBRL) and 1 volume chloroform-isoamyl (24:1), once with a half volume phenol and half volume chloroform-isoamyl (24:1), and once with 1 volume chloroform-isoamyl (24:1). Vector DNA was ethanol-precipitated, washed with 70% ethanol and resuspended to 0.1 μg/μL in ddH_2O (about 20 μL).

PCR products were prepared for insertion into the Kpn I cut M13 vector by purification, digestion with Kpn I and repurification from an agarose gel. PCR products

were extracted twice with phenol volume chloroform-isoamyl (24:1), once with a half volume phenol and half volume chloroform-isoamyl (24:1), and twice with 1 volume chloroform-isoamyl (24:1). Purified DNA was precipitated, washed with 70% ethanol and dissolved in 15 μL TE. The purified DNA was incubated in 2 volumes ddH_2O, 10X OPA (One-Phor-All PLUS buffer) to 1X concentration and 45 units Kpn I (Pharmacia) for 2 hours at 37°C. To purify Kpn I cut PCR product from enzyme and other reactants the insert preparation was applied to a 2% NuSieve agarose (FMC BioProducts)/1X TAE gel. The excised DNA band of interest was purified from the agarose via phenol, phenol/chloroform and chloroform extraction and ethanol-precipitated. Pellets were washed with 70% ethanol and resuspended in 20 μL ddH_2O; the DNA was then ligated into the Kpn I cut M13 vector and cloned.

Ligation reactions, including positive and negative controls, were performed in three ratios of vector to insert, using 0.01 μg, 0.03 μg and 0.003 μg of prepared vector. The 20 μL reactions also contained 3 μL insert prep (regardless of concentration), ligation buffer to 1X concentration and 4 Weiss units of ligase (Pharmacia). Ligation reactions were transformed into MAX Efficiency DHαF'IQ™ Competent Cells (GibcoBRL): 0.5 μL ligation reaction, or 1 μL M13mp19 control DNA was gently mixed into 36 to 39 μL competent cells, incubated 30 minutes on ice, heat shocked for 45 seconds at 42°C and incubated 2 minutes on ice. Once cells were transformed 0.9 mL SOC medium, 100 μL DH5α *E. coli* lawn cells and 3 mL 45°C YT top agar (containing 0.2 mg/mL XGal and 0.02% IPTG) was added, and the cell mixture was plated on YT agar plates and incubated overnight at 37°C.

M13 Template Preparations. Vectors containing the insert DNA were purified from the bacterial cells via template preparation. A starter culture of 1 mL DH5α *E. coli*

lawn cells was inoculated into 5 mL SOC medium (containing 8 M $MgCl_2$) and shaken for 4 hours at 37°C. Two mL terrific broth (TB) containing 10 μg/mL kanamycin (kanamycin sulphate, USB) and 0.6% starter culture was inoculated with a plaque containing the insert DNA and grown overnight with shaking at 37°C. Glycerol stocks of overnight cultures were made and stored at -70°C. Vector/insert DNA was extracted from the bacterial cells. DNA was precipitated at room temperature from 1.2 mL of the supernatant with 250 μL 20% PEG/2.5 M NaCl (final concentration is 3.4% PEG [polyethylene glycol, Sigma], 430 mM NaCl). The pellet was resuspended in 100 μL TE (pH 8.0), extracted with phenol, then chloroform-isoamyl and ethanol-precipitated. The purified DNA was washed with 70% ethanol and resuspended in 20 μL TE, pH 8.

2.3 Ancient DNA Methodology

Precautions taken with ancient DNA. Many precautions were taken to ensure the authenticity of DNA sequences recovered from archaeological and museum specimens. To ensure that ancient bone samples were not contaminated with dog hair derived from researchers' clothing, a blood sample from S. Crockford's malamute (table 2.1), the only member of the research team who owned a dog, was among the first to be sequenced with the contemporary samples. Bone samples were prepared for DNA extraction by Crockford in a separate building. In the laboratory, all glassware dedicated to ancient DNA work was thoroughly washed, bleached and autoclaved. Small batches of solutions were made up frequently, filter-sterilized and used exclusively for ancient DNA. Disposable products were used wherever possible, and pipet tips were used only once. Pipetmen used were dedicated to ancient DNA work. If contamination were suspected, the suspect solution or item was discarded (or decontaminated in the case of glassware, pipetmen, etc.). To reduce risk of contamination between samples, only one individual

was extracted at a time, with a negative control blank extraction. Gloves were changed frequently and were always changed between handling dog and blank samples. A face mask, gloves and a laboratory coat (dedicated to ancient DNA use) were worn when handling anything used for ancient work. The work was performed in a laminar flow hood, which was cleaned with 70% ethanol and UV irradiated before each procedure commenced. Multiple PCR negative controls were prepared in addition to control extractions. Taking all of these precautions permits confidence in the resulting DNA sequences from ancient specimens.

Ancient DNA Extractions. The museum skins were extracted using the CTAB method modified (McArthur 1996; Byun *et al.* 1997) from Doyle and Doyle (1987). Approximately 20 mm^2 of tissue was minced and ground with a pinch of sterilized quartz sand in 400 μL of preheated (65°C) CTAB buffer (100 mM Tris-HCl, pH 8, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 0.2% ß-mercaptoethanol) and incubated at 65°C for 2 or more hours with frequent vortexing. Liquefied tissue was extracted with 1 volume chloroform-isoamyl (24:1). DNA was precipitated from the aqueous layer with 1.5 volumes cold 70% ethanol and 1/16 volume 3 M NaOAc, pH 5.2, washed with 70% ethanol and resuspended in 22.5 μL ddH_2O and 2.5 μL TE, pH 8.0.

One method was used to prepare archaeological bones for extraction, and three methods were used to extract DNA from the bones. The exteriors of the bone samples were sterilized with bleach (Sykes 1993). One to two grams of bone powder were then collected in disposable sterile 15 mL tubes by drilling small holes with a low-speed hand drill. The drill and bit were thoroughly cleaned between samples (Purdue & Patton 1992). DNA was extracted from bone samples using Persson's (1992), Richards *et al.* 's (1995) and Thomas *et al.* 's (1990) protocols.

Persson's (1992) bone extraction method was used on dog, wolf and tuna bones.

Bone powder (0.5 g) was incubated with agitation at 37°C overnight in 8 mL digestion buffer (10 mM Tris-HCl, pH 8.0, 2.0 mM EDTA and 10 mM NaCl) and 4.0 mg collagenase (type 1A-S, Sigma). Samples were centrifuged 5 minutes at 3,000 rpm, and bone was resuspended in 2 mL phosphate buffer (2.5 M K_2HPO_4 neutralized to pH 7 with phosphorous acid). After incubation at room temperature for 15 minutes samples were centrifuged 10 minutes at 3,000 rpm, supernatant collected and the incubation, centrifugation and collection repeated. Supernatant was concentrated in centricon-30 tubes (Amicon) to 50 μL, diluted to 2.0 mL and reconcentrated. DNA was precipitated from the final concentrate with 0.08 volumes of 1.0 M spermidine tetrahydrochloride (Sigma) at 4°C for a minimum of 4 hours. The pellet was dissolved in a high salt solution, i.e. 10X PCR buffer (100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 500 mM KCl). To remove possible PCR inhibitors from some of the DNA extracts (Hagelberg & Clegg 1991; Höss *et al.* 1996) the Wizard DNA Clean-up kit (Promega) was used, as suggested by Richards *et al.* (1995). Extract was added to 1 mL of Wizard Clean-up resin and applied to the kit purification column. The column was washed with 80% isopropanol, and DNA was eluted with 50 μL prewarmed (65-70°C) ddH_2O. For samples which still did not yield amplifiable DNA, a 10% Chelex-100 (Bio-Rad) solution was added to 5% final concentration and incubated at room temperature for 10 minutes (Richards *et al.* 1995).

Another method used to extract DNA from archaeological bones was that of Richards *et al.* (1995). Approximately 1.0 g of bone powder was rinsed twice with ddH_2O. Bone powder was incubated in 5 mL 0.5 mM EDTA pH 8.0 at 37°C for 24 hours. Sarkysol was then added to 0.5% and Proteinase K (Boehringer Mannheim GmbH) to 100 μg/mL and incubated an additional 24 hours at 37°C. Supernatant was extracted with phenol, phenol/chloroform and chloroform-isoamyl (24:1). Extracts were desalted and concentrated with centricon-30 tubes and purified from EDTA with the Wizard DNA

Clean-up kit (Promega). Extracts were treated with 10% Chelex as outlined previously.

The Thomas *et al.* (1990) method also was used to extract DNA from archaeological dog and wolf bones: 0.5 g of bone powder was digested in 8 mL buffer (10 mM Tris-HCl, pH 8.0, 2 mM EDTA, 10 mM NaCl, 1% sodium dodecyl sulphate (SDS), 10 mg/mL dithiothreitol (DTT) and 0.5 mg/mL proteinase K) for 20 hours at 37°C with constant agitation. The resulting supernatant was extracted twice with phenol and once with chloroform-isoamyl (24:1), and approximately 1/10 of the extract was concentrated in a Centricon-30 tube. DNA extracted using the preceding protocols was amplified using a variety of PCR methods.

Amplification of ancient DNA. Several variations of two PCR methods (appendix A) were utilized to amplify DNA from the ancient extracts. One of the two most commonly used methods was very similar to that used to amplify contemporary DNA. Changes included using 1 μL of undiluted DNA as well as 1 μL of 1/10, 1/100 and 1/1,000 dilutions as template DNA, adding 20 μg/mL of BSA (bovine serum albumin) to the PCR reaction mixture, and increasing the number of cycles from 30 to 35. The second method was slightly modified from Thomas *et al.* (1990): 50 μL reactions contained 67 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 10 mM ß-mercaptoethanol, 250 μM of each dNTP, 2 μg BSA, and 2 to 5 units of *Taq* polymerase (Pharmacia and GibcoBRL). Samples were denatured at 94°C for 4 minutes, cycled 40 times through 30 seconds at 94°C, 1 minute at 50°C, 1 minute at 72°C and extended for 7 minutes at 72°C. All ancient DNA PCR reactions were carried out in the Perkin Elmer 9600.

Primers for ancient canid DNA PCR reactions were designed to amplify a 200 bp (primers 43 and 45) and a 300 bp (primers 13 and 15) fragment through the most informative (hypervariable) region of the d-loop. Primer sequences are: primer 15, 5'-ACTATTCCCTGACACCCCTACATT- 3' (position H15942), primer 13, 5'-GGCCC GGAGCGAGAAGA-3' (L15682), primer 45, 5'-TATATTGAATCACCCCTACT-3'

(H15920), and primer 43, 5'-AAGCCCTTATTGGACTA-3' (L15745). A primer, Var 100 Fwd 5'-YYTTACATAGGACATAT-3' (H15754), also was designed to act with primer 43 to amplify a 100 bp region from undetectable amounts of the 300 bp PCR product, but was ineffectual. To retrieve additional downstream sequence information from the ancient samples, a two-stage amplification reaction was performed. Primers L651 and H15980 (for 1,100 bp product) were used in the first amplification reaction, followed by a second reaction with primers L650 and the Internal Fwd primer, located at H15538 (5'-ATGGGACATCTCGATGGACTA-3'). The primers cyt bL and cyt bH for archaeological tuna were taken from Bartlett and Davidson (1991) to amplify the cytochrome *b* gene of the mitochondrial genome.

Two common problems encountered with DNA extracted from ancient tissues are the inability to amplify the DNA and contamination of the PCRs. As many DNA extracts were difficult to amplify, several modifications of the ancient DNA PCR methods (appendix A) were attempted. These included altering the amount of BSA (0-50 μg), utilizing different concentrations (0.02, 0.04 and 0.1 units/μL) and brands of *Taq* polymerase (Pharmacia, Gibco, Perkin-Elmer), altering primer concentrations (0.25, 0.5 and 1.0 μM), and using different PCR buffers (see Appendix A). Changes also included altering the number of cycles within the PCR programme, as well as the duration of some of the steps (see appendix A). Whenever contamination was detected, solutions were discarded and new ones prepared. Measures that were taken when PCR contamination persisted included the use of solutions prepared by others in separate laboratories and those that were prepared commercially. Several water sources and methods of water purification (autoclaved ddH_2O, filtered ddH_2O, filtered and autoclaved ddH_2O) were also used. When contamination could not be eliminated, nor its source (or sources) determined, the dog extract, control extract and PCR negative control all were sequenced. Sequences which were present only in the dog extract were considered to be authentic ancient DNA and were

confirmed by subsequent extraction, PCR, cloning and sequencing. Contamination and difficulties in amplification were recurrent problems.

TA cloning. TA cloning (Invitrogen) was used for the ancient DNA PCR products because of its high efficiency with small DNA fragments. The PCR products were excised from 2% NuSieve agarose gels, and to the melted slices was added 1 mL of resin. The solution was applied to Wizard Prep columns (Promega), which were washed with 80% isopropanol, and DNA was eluted with 35 µL ddH_2O. Two µL of the purified DNA were ligated into the TA cloning vector and incubated at 12-14°C overnight (12-18 hours). The 10 µL ligation reaction also consisted of 1X ligation buffer, 50 ng pCR™II vector and 4 Weiss units of T4 DNA Ligase. Vector with ligated PCR product was transformed into One Shot™ INVαF' cells (Invitrogen). To each tube of competent cells 2 µL 0.5 M ß-mercaptoethanol and 1 µL ligation reaction were added, mixed gently and incubated on ice for 30 minutes. Cells were heat shocked for 30 seconds at 42°C and then placed on ice for 2 minutes. After the addition of 450 µL of SOC, cells were shaken at 225 rpm for 1 hour, and then 50 and 75 µL of each reaction were plated onto LB plates (containing 60 µg/mL ampicillin and 50 µg/mL X-Gal) and incubated overnight at 37°C.

Plasmid Mini Preparations. The plasmid mini preparation procedure was modified (Byun pers. comm.) from Titus (1991). Six mL of Terrific Broth (TB), containing 10 mg/mL ampicillin (Sigma), was inoculated with a bacterial colony and shaken overnight at 37°C. Glycerol stocks were made before centrifugation. Liquid was decanted and pellet were resuspended and incubated at room temperature for 5 minutes in 200 µL ice-cold cell-lysis buffer (25 mM Tris-HCl, pH 8.0, 10 mM EDTA, 50 mM glucose). Two volumes fresh 0.2 N NaOH/1% SDS solution were added and incubated another 5 minutes. After addition of a half volume of ice-cold potassium acetate solution, pH 4.8 (3 M potassium, 5 M acetate) samples were incubated 5 minutes on ice.

Supernatant was digested with 20 μg/mL DNase-free RNase A (Sigma) for 1-2 hours at 37°C, and then phenol, phenol/ chloroform-isoamyl (24:1) and chloroform-isoamyl (24:1) extracted. DNA was precipitated with 2.5 volumes 95% ethanol, washed with 70% ethanol and resuspended in 16 μL ddH_2O. The DNA was reprecipitated with 5% PEG and 0.5 M NaCl for 20 minutes on ice, washed with 70% ethanol and resuspended in 13 μL ddH_2O

2.4 Automated Sequencing

DNA from all individuals was sequenced from at least three clones on the ABI automatic sequencer. Sequencing reactions were prepared according to the ABI dye primer cycle sequencing protocol (1995). Template preparations (with a 260/280 ratio of 1.8 or higher) were diluted to 100 ng/μL and plasmid preparations (ratio 1.5 or greater) to 250 ng/μL. One μL of the DNA was added to A and C PCR tubes and 2 μL DNA to G and T tubes. Four μL of A and C FS *Taq* (or CS *Taq*) ABI PRISM™ mix and 8 μL of G and T PRISM™ mix were added to their respective tubes. The cycle sequencing programme was: 2 minute denaturation at 95°C, 30 cycles of 15 seconds at 95°C, 1 minute at 55°C, 1 minute at 70°C, 30 cycles of 15 seconds at 95°C and 1 minute at 70°C. Samples were precipitated by adding 100 μL 95% ethanol and 2 μL 3 M NaOAc, pH 5.2 to A tube, which was sequentially transferred to the other PCR tubes until all nucleotides were combined for each sample. Pellets were dissolved in 4.1 μL formamide/EDTA (50 μL formamide and 10 μL 50 mM EDTA, pH 8) shortly before being loaded on the sequencing gel. Samples were denatured at 90°C for 3 minutes immediately before being loaded on 4% (for short sequences) or 5% (for longer sequences) polyacrylamide gels. Sequencing reactions were run on the ABI 373A and ABI 377 (Perkin Elmer) automated sequencers.

All contemporary canid sequences were elongated to 720 bp using the Internal Fwd

primer and the ABI PRISM™ dye terminator cycle sequencing kit with AmpliTaq® DNA polymerase, FS, according to the manufacturer's protocol (1995). Five-hundred ng of template preparation, 3.2 pmol of the primer Internal Fwd and 8.0 μL of terminator ready reaction mix (containing PCR buffer and dNTPs) were included in a 20 μL reaction. The thermal cycling programme consisted of 10 seconds at 96°C, 5 seconds at 50°C and 4 minutes at 60°C, for 25 cycles. Unincorporated dye terminators were removed from extension products by a 10-to-15-minute ethanol-precipitation (74 μL 70% ethanol with 0.5 mM $MgCl_2$).

2.5 Analysis of Sequence Data

Raw sequence data were analysed on ABI PRISM™ DNA Sequencing Software, version 2.1.0 (Perkin Elmer). A minimum of three sequences were assembled to obtain a consensus sequence for each sampled individual (SeqMan, Mac version 3.04 DNASTAR). Consensus sequences were downloaded to EditSeq, Mac version 3.83 (DNASTAR), and aligned with MegAlign, Mac version 3.03 (DNASTAR), invoking the clustal V option. The alignment was exported to PAUP 3.1, visually checked for accuracy, and analysed for phylogenetic inference. Phylogenetic analyses also were performed with PAUP* 4.53 (Mac and Unix platform).

Phylogenetic trees were inferred using parsimony (PAUP, PAUP*), neighbour-joining (PAUP*) and UPGMA (PAUP*) algorithms. Searches for the most parsimonious tree utilized informative characters only. Characters were unweighted, since the d-loop is a noncoding region, and gaps were scored as single characters (regardless of length). A majority-rules consensus tree was estimated from 5,000 most parsimonious trees using the heuristic search method. Bootstrapping of 5,000 trees was used to evaluate confidence in the resulting clades (only values greater than 50 were retained). Jukes & Cantor's (1969) one parameter model and Kimura's (1980) two parameter model were

used to compute genetic distance between taxa for NJ and UPGMA methods. Gaps were not included in the distance methods.

Results

3.1 Success of Methodologies

At the commencement of this project no standard methods existed for extracting DNA from ancient tissues; therefore, a significant part of this study involved finding ancient DNA methods that would work consistently. Several methods for recovering DNA from ancient bone were tested and modified: Thomas *et al.*'s (1990) method was found to be the most successful in this study. The modified CTAB method (McArthur 1996; Byun *et al.* 1997) worked well for the museum skins, as was verified by other studies in the Koop laboratory (e.g. McArthur 1996; Byun *et al.* 1997).

As expected, overall success with the ancient tissues was quite low (tables 3.1 and 2.3). This type of work is troublesome because ancient DNA is normally degraded and is often extracted in the company of contaminants and PCR inhibitors (Hagelberg & Clegg 1991; Höss *et al.* 1996). Of the twenty-nine ancient DNA extractions recorded in table 3.1, five (17%) were clean (i.e. no DNA in the control extraction), five others (17%) were clean but there was DNA in the PCR negative control and nine (31%) had DNA in the control extraction as well as in the PCR negative control (see table 3.2). Occasionally, more DNA was amplified from the ancient extract than from the control extract (and PCR negative where applicable). Of seventeen ancient samples, DNA was reproducibly extracted, amplified, cloned and sequenced from five individuals. The sequence data from the five individuals were subjected to phylogenetic analysis.

Museum skin DNA extractions. DNA extractions from museum skins were much more successful than those from bone materials, although some problems also occurred with the skins. On three separate occasions DNA was extracted from one skin in particular (Tahltan 5027); however, sequence analysis showed the presence of

contaminating dog, wolf and coyote DNA. There were several sequences which may have been authentic ancient DNA, as they were similar to other Tahltan samples, but the results were not reproducible. The inability to identify some of the source individuals of exogenous DNA within the Tahltan 5027 extracts suggests at least two possible scenarios. The DNA in the skin was so degraded that many PCR errors were included so that several sequences with random differences were obtained from this individual (Thomas & Pääbo 1993). Alternatively, the skin may have been contaminated during collection, handling or storage (Pääbo 1993), and this would account for the occurrence of wolf, coyote and dog haplotypes (e.g. dust particles from more recently collected and prepared canids, etc.). Given the sensitivity of the PCR method, the likelihood that the DNA in the skin sample was degraded, and that some haplotypes could be traced to individuals that had been previously sequenced in the laboratory, it is possible that DNA which had been present in the laboratory for one to three years was amplified as well. Normally, one would not detect this minuscule amount of exogenous DNA in the presence of a large number of fair to good quality ancient DNA molecules since the DNA template that is present in higher concentration is preferentially amplified (Thomas & Pääbo 1993). It seems logical that DNA molecules which are at most three years old would be preferentially amplified over a few severely degraded 60-year-old molecules. DNA from Tahltan bear dog skins 2892 and 4758 appeared to be less degraded since these samples yielded DNA which was successfully and reproducibly extracted, amplified, cloned and sequenced (see table 3.1). Although ancient DNA is difficult to obtain, usable fragments of DNA were extracted from two of the three Tahltan bear dog samples.

Archaeological bone DNA extractions. DNA was more difficult to extract from archaeological bone than from museum skin in the current study. Several of the bone samples did not yield amplifiable DNA: wool dogs 17, 65 and 68; village dogs 19, 72 and 73; prehistoric dogs 30 and 31; Vancouver Island wolves 53 and 56, and bluefin tunas 34

and 35 (see table 3.1). Bone samples that were reproducibly extracted, amplified, cloned and sequenced were historic dog 41, wool dog 64 and village dog 74 (see tables 3.1 and 2.3). The differences in extractability of DNA from museum skin versus archaeological bone could be attributed to the difference in age (about 60 years versus 200 to 4,000 years), preservation conditions, or perhaps a combination of these reasons. Dried untanned skins will often yield amplifiable DNA (Thomas & Pääbo 1993), but when both tissues are nearly equivalent in age, DNA has been found to be more readily obtained from bone than skin (Cooper *et al.* 1992). It is possible that the hard bone matrix protects the DNA from several degradation processes, such as oxidation and hydrolysis (Lindahl 1993a). Even though many difficulties were encountered in extracting DNA from archaeological bone, three samples yielded usable DNA.

Amplification of ancient DNA extracts. The success rate in amplifying "clean" PCR products from ancient tissue extracts was very low (table 3.2). A clean PCR product is the result of a PCR reaction in which no DNA was amplified in the PCR negative controls. One hundred and ten PCR reactions were performed on twenty-nine ancient DNA extracts and their corresponding control extractions (table 3.1). Of these, fifty-three reactions (48%) yielded no amplified DNA, forty reactions (36%) were contaminated (i.e. there was DNA in the PCR negative controls) and seventeen reactions (15%) were clean. At least twenty-four other PCRs from Tahltan extracts were performed by others in the laboratory, but all were contaminated (S.A. Byun & U. Rink, pers. comm. 1997). Of the many PCR methods used (see Appendix A), that of Thomas *et al.* (1990) worked most consistently.

Authentication of ancient DNA sequences. One of the major problems with research projects that utilise ancient DNA techniques is verifying the authenticity of ancient DNA. Confidence can be exerted in the results of this study because they are novel (these sequences were not found in any other known samples). When the indigenous dog

DNA sequences are compared to those of other dogs and wolves, all of the confirmed ancient sequences contain unique nucleotide sites. The sequences were reproduced from an additional independent DNA extraction. With independent extractions it is therefore unlikely that these sites can be attributed to PCR replication errors. Although contamination of ancient DNA with contemporary DNA is always a concern, unique sequences and similarities between several independent samples support the authenticity of the indigenous dog ancient DNA.

It is also unlikely that the DNA sequences were derived from nuclear insertions. Nuclear insertions are low in copy number and therefore not likely to be amplified unless the PCR primers preferentially amplify these insertions (Kringer *et al.* 1997). The PCR primers were designed to be highly specific (Kringer *et al*. 1997) to dog and wolf d-loop DNA; assuredly then, the amplified DNA originated from the ancient dogs' mitochondrial genome, rather than from a nuclear copy of a modern contaminant.

3.2 Sequence Data

A minimum of 718 base pairs (bp) were sequenced from forty-eight contemporary canids, and a 305 bp segment through the most variable region was sequenced in the five ancient canids. A consensus sequence from each individual sampled was obtained from a minimum of three clones (except in the case of akita 23, for which only two clones were obtained), and all of the consensus sequences were aligned with each other and compared. The entire 1 200 bp PCR product covering the d-loop was sequenced from one individual (whippet 13), and sequences up to 900 - 1,000 bp were obtained from several other individuals. Comparison of the sequence data to assess suitability for phylogenetic analysis revealed the presence of tandem repeats (fig. 3.2) at the 3' end of the canid sequences. Because of the rapid mutation rate within these heteroplasmic repeats (Hoelzel *et al.* 1994), this region of the sequences could not be aligned with confidence. This

confirms the data of Okumura *et al*. (1996) and other authors who have reported these hypervariable tandem repeat units in the d-loop of dogs and bears (Rothuizen 1996), several other carnivores (Hoelzel *et al.* 1994) and teleost fish (Lee *et al.* 1995). For this reason the study was limited to the 5' 718 bp of the d-loop, thus excluding the tandem repeats from the analyses. Subsequent analyses were based on the results of the multiple alignment of all nucleotide sequences (fig. 3.1).

Nineteen heteroplasmic polymorphic sites were found in the 718 bp multiple sequence alignment (fig. 3.1). One of these polymorphisms is found in several individuals (site 9), and two occur in two individuals (sites 211 and 226), but most occur in only one individual. The occurrence of heteroplamsy is not unexpected, as the rapid mutation rate in the d-loop and the high number of mitochondria within the cell can result in two or more distinct mitochondrial haplotypes within the same cell (Avise *et al.* 1987; Avise 1991). Multiple haplotypes in the same individual would then be passed on by females through gametic cells. This condition is thought to be evolutionarily short-lived as homoplasmy is quickly reverted to via genetic drift and gametic sampling (Avise 1991; Avise *et al.* 1987).

To estimate phylogenetic relationships among dogs and the other canids in this study, three methods were applied: UPGMA (unweighted pair group method with arithmetic mean), NJ (Neighbour Joining) and maximum parsimony. All sequence information generated by this study and that of Okumura *et al.* (1996) was utilised in the UPGMA (fig. 3.3) and NJ (fig. 3.4) distance methods. UPGMA assumes a molecular clock, while NJ minimizes the total length of the tree. The distance data also were used to perform a relative rate test (fig. 3.5). The eighty-six informative sites revealed by the DNA sequence multiple alignment (fig. 3.1) were utilised in a maximum parsimony phylogenetic analysis of the data (fig. 3.6). All three methods demonstrated the close relationship of dogs and wolves: several groups within the dogs and wolves were differentiated but were not statistically well supported.

Analyses were performed with all of the long and short sequence data to determine the phylogenetic affinities of the extinct North American indigenous dogs. Once this was achieved the analyses were re-executed with only the 718 bp sequences in order to eliminate skewness in the results. The skewness (i.e. inflated distance values between short and long sequences) was a result of using short sequences through the most variable portion of the d-loop, the hypervariable region. Within the trees, the positions of the extinct North American indigenous dogs were estimated based on the phylogenetic positions of their closest relatives. In another parsimony analysis, all canid sequences were restricted to 305 bp; however, this approach did not allow definition of any groups within the dogs and wolves (data not shown).

3.3 Distance Analyses

Analyses using UPGMA and NJ distance methods resulted in topologically similar trees (figs 3.3 and 3.4). All distance values were corrected for superimposed substitutions. The two main models used to correct the data included Jukes-Cantor one parameter model (Jukes & Cantor 1969) and Kimura's two parameter model (Kimura 1980). Kimura's two-parameter model was more heavily relied upon to compute genetic distances, because it accounts for a higher rate of transitional mutations than transversional ones, as is found in the d-loop (see table 3.4 and Kimura 1980). The major phylogenetic groups were the same with both models, but relationships within these groups were somewhat variable, as discussed below.

Okumura *et al*.'s (1996) data were included in the analyses. In subsequent discussions, the dog data from Okumura *et al.* (1996) will be referred to as the "Japanese dogs", even though not all of these dog haplotypes represent indigenous Japanese breeds (table 3.3).

UPGMA. Six major clusters were differentiated in the UPGMA phylogenetic

tree: 1) foxes, 2) coyotes, 3) wolves, 4) two groups of ancient dogs [(a) Tahltan bear dog, Coast Salish village dog and historic dog and Yukon wolf 22 and (b) Coast Salish wool dog and several Japanese dogs], 5) modern dogs and 6) Norwegian elkhound. Another minor cluster of two Japanese dogs is found outside the modern dogs (D83611 and D83637). A summary of genetic distances among these major groups can be found in table 3.5, and between pairs of individuals in appendix B. To avoid biases contributed by shorter sequences obtained from the most variable region, only full sequences (i.e. 718 bp) were used to determine the relationships of the six major groups to each other.

A clear separation of the foxes from the coyotes, wolves and dogs is observed in fig. 3.3. Coyotes also formed a distinct group that split off from wolves and dogs, thus confirming that the indigenous North American dogs were not derived from coyotes as some early European explorers hypothesized. On average, the fox differed from both the coyote and wool dog (C) clusters by 17.2% and from all other dog clusters and the wolf (A) cluster by 17.1%. The two foxes, one from the Yukon and the other from Japan, varied from each other by 1.6%. Coyotes differed from both the wool dog (C) and elkhound (D) clusters by 6.2%, from the modern dog (E), Japanese dog (F), wolf (A) and other North American indigenous dog (B) cluster by 6.1%. Within the coyotes, genetic variation was 1.0%. According to estimates from the fossil record, coyotes and wolves diverged one million years ago (Wayne *et al.* 1991). Fig. 3.3 demonstrates that foxes and coyotes were clearly distant to all of the dogs and wolves.

As can be seen from the UPGMA tree (fig. 3.3), the wolves do not partition according to subspecies. Divergence within the "wolf" cluster (A) ranges from 0 to 1.7%, and the group as a whole differs from the modern dogs (E) by 2.0%, from the North American indigenous dogs (B) by 1.2%, from the wool dog (C) by 2.1%, from the elkhound (D) by 1.7% and from the Japanese cluster (F) by 2.2%. Several Japanese dogs are included in the "wolf" cluster (A): D83601, D83604, D83607, D83619, D83620,

D83622, D83625, D83632 and D83634. This indicates that either a domestication event or dog-wolf hybridization has occurred in Japan. Within the wolf cluster, the most distantly related wolf is Yukon wolf 2, which groups by itself at the outer edge of the cluster. The second most distant individual is Japanese dog D83632. Inside of these deeper branches are two shallower branches consisting of (1) Arctic wolf 1, European wolf 1 and eight closely related Japanese dogs, (2) European wolf 2, Vancouver Island wolf 3 and Yukon wolves 5 and 6. Nonpartitioning according to subspecific status may be attributed to either common ancestry or to the translocation of wolves by humans. The mitochondrial haplotypes are clearly more widely dispersed than would be expected from natural subspecies.

The extinct indigenous dogs fell into two clusters, the "North American ancient dog" cluster (B), containing the Tahltan bear dogs, Coast Salish village dog and historic dog as well as Yukon wolf 22, and the "wool dog" cluster (C) containing the Coast Salish wool dog and several Japanese dogs. The two clusters differed from each other by 2.2%. The wool dog cluster (C) differed from modern dogs (E) by 1.7%, from the elkhound by 2.2% (D), and from the Japanese cluster by 1.9% (F). The other indigenous dogs (B) differed from these clusters by 1.2%, 1.7% and 1.9% respectively. Within the North American indigenous dog cluster (B), the two Tahltan bear dogs, Coast Salish village and historic dogs are placed with Yukon wolf 22, which groups closely to Yukon Wolf 4. This cluster is placed interior to the wool dog cluster (C) which is at the outer edge of the wolf cluster. Within the wool dog cluster (C), the wool dog, D83624, D83636 and D83638 are 100% similar. The grouping of Yukon wolves 22 and 4 with the indigenous dogs suggests a common ancestor, as does the clustering of the wool dog with some of the Japanese dogs. The wool dog and cohorts seem to retain an ancient haplotype from a domestication or introgression event in Asia. The other North American indigenous dogs may retain the mitochondrial haplotype of prehistoric wolf populations from which the native dogs were

domesticated; although determining whether the dogs were domesticated in Asia or North America is not possible with the current data set. All other evidence to date indicates that dogs were brought to North America by early human immigrants as fully domesticated animals (Lawrence 1967, 1968; Olsen & Olsen, 1977; Olsen 1985; Morey & Wiant, 1992). Conversely, the two Yukon wolves could represent a population that descended from North American indigenous dogs which interbred with prehistoric wolves (i.e. ancient introgression).

Of all the modern dog breeds examined, only the Norwegian elkhound (cluster D) maintains a unique mitochondrial signature. The elkhound branches well outside of the wolf cluster and differs from the other dogs by 2.2%, while divergence within the other modern dogs is only 0.5%. The elkhound differs from the minor Japanese dog cluster (F) by 2.1%. If the elkhound is the sole survivor of a unique mitochondrial lineage, the uniqueness may have resulted from a combination of the breed's status as the national dog of Norway and its isolation in that country until this century (Glyn 1967; Wallo 1987).

Despite tremendous morphological and behaviourial differences, none of the modern breeds (cluster E) examined are genetically distinct. Three major groups formed within the modern dog cluster (E). The inner cluster is comprised of two Vancouver Island wolves, all whippets, one maremma and several indigenous and non-indigenous Japanese dogs. Divergence within this group ranges from 0 to 0.3%, whereas divergence within the middle cluster of dogs ranges from 0 to 0.4% and within the outer major group it ranges from 0 to 0.6%. The middle cluster includes eight dog haplotypes from the present study (a greyhound, two malamutes, a Siberian husky, two maremmas and both great Pyrenees), the two dogs obtained from the GenBank database and three indigenous and one non-indigenous breeds of the Japanese dogs. The outer cluster contains five dogs from the present study (the shar-pei, both samoyeds, one malamute and one akita) as well as the majority of the indigenous Japanese dogs and some non-indigenous Japanese dogs.

Outside these three major groups of modern dogs, there are two Japanese dogs, D83629 and D83635, which are closely related to each other, but differ from all other breeds by 0.6%. Siberian husky 11 differs from all the dogs by 0.7% and is at the outer edge of the dog cluster. Outside the dog cluster are two Japanese dogs (cluster F), one indigenous (D83611) and one non-indigenous (D83637), which are closely related to each other but differ from the other dogs by 1.4%. Although three major clusters of dogs were found in the UPGMA tree, they were all closely related, with a maximum divergence of 0.7% (or minimum similarity of 99.3%) within the larger dog cluster. Although all of the dog samples collected for this study were from registered purebreds, it must be pointed out that many dog breeds that are registered with the North American and British Kennel Clubs have been recently crossed with other breeds to introduce new characteristics to “improve” the breed. Some of these crosses have not been documented.

An interesting result that was found within the dogs is evidence of recent wolf/dog introgression. Two of the three Vancouver Island wolves are probably a result of a recent introgression event because they were very closely related to some dogs (0 to 0.1% difference) and were at maximum 0.7% different from the other dogs. Conversely, these two Vancouver Island wolves differed from other wolves by 2.0-2.1%. This finding substantiates earlier morphological evidence of introgression on Vancouver Island (Friis 1985). It cannot be determined if the northern Vancouver Island introgression is a result of female dogs wandering into the wild and raising her pups there, or if a female was mated ‘at home’ (either accidentally or purposefully), and the hybrid offspring went feral. It has been suggested that such hybrids could be more prone to becoming nuisance animals and therefore more likely to become part of a study such as this one, through donations from wildlife conservation officials (Crockford pers. comm.).

Neighbour Joining. The composition of major clusters found in the Neighbour Joining (NJ) tree (fig. 3.4) are essentially the same as those found in the UPGMA tree (fig.

3.3). Differences between the two trees include placement of the Norwegian elkhound (D) and the placement of the main clusters within the modern dog cluster (E). As with the UPGMA tree, both the foxes and coyotes are clearly distinct from the large dog-wolf cluster, differing from each other by 15% and from the dog-wolf cluster by approximately 16.9% and 7.5% respectively. Genetic distances vary somewhat between the two trees, but generally the distances between the clusters of the NJ tree correspond with those in table 3.5, especially among the wolf and dog clusters.

As found in the UPGMA tree, in the NJ tree the wolf cluster (A) is composed of two subclusters containing (1) Arctic wolf 1, European wolf 1 and eight Japanese dogs, (2) European wolf 2, Yukon wolves 5, 6 and 2 and Vancouver Island wolf 3. There is also a Japanese dog, D83632, within the wolf cluster. Variation within the wolf cluster ranges from 0.1 to 1.6%. Outside the wolf cluster, the North American indigenous dog cluster (B) is found, and outside these two clusters is the wool dog cluster (C).

Within the dog cluster there are three large clusters and two smaller ones. Again, Vancouver Island wolves 1 and 2, the whippets, a maremma, two malamutes and several Japanese dogs are within the inner cluster. In the middle cluster are found a greyhound, Siberian husky, two maremmas and four Japanese dogs. In the outer large cluster are the two akitas, both great Pyrenees, the two samoyeds, a malamute, a Siberian husky and six Japanese dogs. Outside these larger clusters are two smaller clusters which consist of (1) the two sequences obtained from the GenBank database and (2) the shar-pei and three Japanese dogs. Outside this dog cluster (E) is the dichotomy of D83611 and D83637 (cluster F), as was also found in the UPGMA tree. Divergence within the dogs ranges from 0 to 0.6% and averages 0.5%.

The major difference between the NJ tree and the UPGMA tree is the branching of the elkhound outside the wolves and dogs (rather than between the wolves and dogs as it was in the UPGMA tree). The elkhounds differ from both the modern dogs (E) and

wolves (A) by 2.0% and from the North American indigenous dogs (B and C) by 1.9%. Generally though, the topology of the UPGMA and NJ trees is similar.

NJ tree bootstrap values. To measure confidence in the branching patterns of the NJ tree, bootstrapping was employed. The bootstrap method works by resampling from the original data set creating a series of pseudosamples, the sampling variance is then estimated from these repetitions (Swofford *et al.* 1996). Most of the major clusters are supported by bootstrap values greater than 50% (fig. 3.4), with the exception of the wolf cluster (A). The fox and coyote clusters are very well supported by bootstrap values of 100 and 99 respectively. Some groupings within the wolves are marginally supported (fig. 3.4) but in general the wolves are not well supported as a distinct cluster. Both North American indigenous dog clusters are well supported, by a value of 97 (or 99 with short sequences) for the wool dog cluster (C) and by 85 (or 71 when the short sequences are included) for the other North American indigenous dogs (B). Most of the modern dogs fall into a cluster which is supported by a bootstrap value of 68. The other two dog clusters, D83611/D83637 (F) and Norwegian elkhounds (D) are supported by bootstrap values of 66 and 100 respectively. The F cluster is supported by a much higher value of 99 when the short sequences are included in the bootstrap analysis. Most of the other bootstrap values decrease when the short sequences are included in the analysis (data not shown).

Relative rate test. The data was used to ascertain if any of the canid lineages were evolving more rapidly than the others. One can determine if two lineages have been evolving at the same or different rates using Sarich and Wilson's (1973) relative rate test. The advantages of the test are that a molecular clock is not assumed and the divergence time of the taxa is not required. Instead, the test uses a reference taxon that is known to have branched off before the two taxa of interest (Sarich & Wilson 1973; Li 1997). The greater distance between fox and dog/wolf than between coyote and fox in the Neighbour Joining tree suggests that the dog-wolf lineage is evolving more rapidly than the coyote lineage.

There was almost no difference in evolutionary rates between the modern dogs and wolves, using both coyote (fig. 3.5a) and fox (data not shown) as the reference species. There is, however, a substantial difference in evolutionary rates between coyote and the dog/wolf cluster, using fox as a reference species (fig. 3.5b). Although there is a significant increase in rate in the dog-wolf cluster, it cannot be determined if domestication causes this increase, because of the complication presented by extensive introgression between the dogs and wolves (Vilà *et al.* 1997).

3.4 Parsimony Analysis

The parsimony analysis relies on the use of informative characters (or sites) rather than genetic distance between sequences. Informative characters are those that are present in more than one individual (or taxon) in at least two states (e.g. long snout versus short snout). Although UPGMA, NJ and parsimony methods are fundamentally different, the major results of all methods were the same. However, there are often multiple equally most parsimonious trees that result from the analysis; in these cases a consensus of these trees is computed. Most of these equally parsimonious trees differed only in the placement of individuals within major clusters. A consensus of 5,000 equally most parsimonious trees of the canid data (fig. 3.6) shows two strongly supported clades (fox and coyote) and six weak clades (wolf, ancient wool dog, ancient hunting dog, modern dog, two modern Japanese dogs and Norwegian elkhound), though no clear distinctions could be made between modern dogs and wolves. The biggest difference between the distance and parsimony methods was the placement of both of the ancient dog groups closer to the modern dogs than to the wolves.

Informative characters. Eighty-six nucleotide sites within the d-loop sequence (fig. 3.1) are informative and can be used for parsimony analysis. Of these eighty-six characters, fifty-six defined the six major groups within the most parsimonious

phylogenetic tree (fig. 3.5). Thirteen of the informative characters can be described as either wolf (n=10) or wild (n=3) character states. A wolf site is one in which a particular base is found within the wolf clade (A) only. A wild site is found in the wolves (A), foxes and coyotes, is often found in Norwegian elkhounds (D) and is rarely found within the modern dogs (E). The DNA sequences of the ancient dogs (B and C) usually contained the wild and wolf categories, but lack information before site 15 and after site 319. The two Norwegian elkhounds share one unique character as well as several others that are found in few other dogs. There are several informative sites within the modern dog clade (E), but none that contribute significant information to the phylogenetic analysis. Based on the information provided by these informative characters, a consensus of the most parsimonious trees was constructed (fig. 3.6).

Maximum parsimony tree and bootstrap analysis. A consensus of the most parsimonious trees shows the partitioning of eight clades: fox, coyote, wolves (A), wool dog and cohorts (C), the other North American indigenous dogs (B), modern dogs (E), Japanese dogs D83611 and D83637 (F), and Norwegian elkhound (D). There are few major differences in topology of the distance versus parsimony trees. Both the fox and coyote are strong clades outside the wolves and dogs, supported by bootstrap values of 100. The wolves, except Vancouver Island wolves 1 and 2 and Yukon wolves 4 and 22, form a clade (A), which is not strongly distinct from the dog clades (B to F). The major difference between the distance methods and the parsimony method is in the placement of the wool dog and cohorts (C) and the other North American indigenous dogs (B) closer to the dogs than to the wolves. The phylogenetic affinities within the wool dog clade are supported by a bootstrap value of 97 and within the other North American indigenous clade by a value of 73. Other differences between the distance and parsimony trees are minor changes of groupings within the modern dog clade (E), a clade that is marginally defined by a bootstrap value of 50. These minor phylogenetic changes will not be mentioned as

bootstrap values are too low to warrant making any inferences about resolution within this clade. The minor clade comprised of Japanese dogs D83611 and D83637 is supported by a bootstrap value of 79, but separated from the modern dogs by a bootstrap value of only 50. The parsimony tree agrees with the NJ tree in the placement of the elkhounds (D) outside both the wolves and dogs. All other bootstrap values that support major branches within the dog/wolf clade are very low (below 50). It can be asserted that dogs and wolves are closely related: the large wolf-dog clade (including clades A to F) is supported by a bootstrap value of 100. Eight clades were resolved within the parsimony tree, but complications introduced by introgression between dogs and wolves limits statements that can be made about the relationships among the clades.

3.5 Summary of UPGMA, NJ and Maximum Parsimony Phylogenies

All three methods of tree reconstruction resulted in similar topology, especially of the major groups, although relationships within these groups varied from tree to tree. The major differences between the UPGMA and NJ trees are found in the closer relationship of dogs and wolves, with the elkhounds falling outside both clusters. To a lesser extent, placement of the three major clusters within the larger dog cluster varied from tree to tree. Although the NJ and UPGMA trees are very similar within the major clusters, the relationships among these clusters differs between the two distance methods. To decide which tree most likely estimates the phylogenetic relationship of dogs to other canids the assumptions of each method must be examined. The UPGMA method assumes that mutations are being accumulated at the same rate across all lineages under examination. Since the relative rate test showed that the dog/wolf lineage is evolving more quickly than the coyote lineage (fig. 3.5), the UPGMA tree is not a phylogenetic tree by definition and, therefore, demonstrates only phenetic differences (Li 1997). However, these phenetic differences could be used to find breed markers among dogs. The NJ method does not

assume a molecular clock, but instead considers rate variation, so this tree is preferred over the UPGMA tree for phylogenetic assessment of the data set. The NJ method reconstructs the phylogenetic tree using a simple algorithm to find a tree with the minimum amount of evolution to explain the relationships of the taxa (Saitou & Nei 1987). The NJ tree is a good visualization of relative distances between individuals and groups, but the maximum parsimony is a better method on which to base evolutionary interpretations of the data. The maximum parsimony method identifies the tree (or trees) that requires the least number of character-state changes to explain the differences among the taxa being studied (Li & Graur 1991). This method is based on a model which assumes that all taxa within a clade are descended from a common ancestor. This method is therefore preferred to determine the evolutionary relationships among dogs and between *Canis familiaris* (extant and extinct) and *C. lupus*.

A recent study by Naylor and Brown (1997) could also prompt one to argue for a preference for parsimony methods over distance methods when studying evolutionary relationships. They demonstrated that collecting more DNA sequence data does not necessarily produce the most accurate estimation of phylogeny. They found that using the most informative regions of the mitochondrial genome (i.e. residues important in protein folding) resulted in a tree that reflected the well accepted interrelationships of nineteen taxa (based on multiple types of data). When they analysed the complete protein-coding region of the genome, an incorrect tree that was well supported by bootstrap values was produced. The argument that more data are not always better can be extended to the preference for parsimony methods over distance methods. Although distance methods use the complete data set, parsimony uses only a subset of the most informative characters to estimate a tree.

3.6 Simple DNA Test to Distinguish Between 'Dogs' and 'Wolves'

Parsimony analysis enabled the identification of specific sites that distinguish

between "dogs" and "wolves". This in turn facilitates the design of a simple restriction enzyme test that can be used in the field to study introgression in hybrid zones. Some restriction enzymes will cut the mitochondrial genome at these wolf/wild sites. The methodology is as follows. Digest DNA with restriction enzymes and apply DNA to 1% agarose gel (most of the fragments will be <400 bp). The enzymes that will cut at or near the wolf/dog diagnostic sites are: Bpu1268 I or Bsc4 I (site 166), Tfl I, HinF III, Taq I, Uba1382 I, Ava III, or Ppu10 I (site 226), BsmN I, BscA I or SfaN I (site 487). Based on the data generated by this study, using these enzymes will result in the following fragment sizes (fig. 3.7). Wolves will display a diagnostic banding pattern of either 375 bp and >644 bp, or of 375 bp, 287 bp and >357 bp fragments. Norwegian elkhound fragments will be 166 bp, 60 bp, 149 bp, 387 bp and >357 bp in size. The rest of the dogs will have one of two banding patterns: 166 bp, 60 bp, 263 bp, 173 bp and >357 bp, or 166 bp, 323 bp, 173 bp and >357 bp. The size of the last fragment depends on the number of repeats found within the individuals. This test would be useful in estimating the extent of introgression between dogs and wolves, giving scientists a preliminary idea of the purity or impurity of a wolf population. One can perform a quick survey to find evidence of hybridizing populations and then analyse those populations in more detail using nuclear markers (including microsatellites). This method would greatly reduce the amount of sampling and laboratory analysis that would otherwise have to be performed for an extensive study of introgression.

The quick test also could have applications to behaviourial biology. It has been suggested that wolf/dog hybrids are more likely to be nuisance animals than purebred wolves and are therefore more likely to be shot and collected for scientific studies (Crockford pers. comm. 1997). The DNA test would allow one to test that theory empirically.

Table 3.1 Success of ancient DNA extractractions and related comments. Samples that were reproducibly extracted and sequenced are denoted by an asterisk (*). Tahltan 2892, Tahltan 4758 and Historic Dog 41/42 were also successfully extracted and genuine ancient DNA sequenced by S.A. Byun. S.A.B. also extracted and sequenced Tahltan 5527, which was contaminated.

Sample	**Bone Type**	**Extraction Method**	**Extraction Successful**	**Comments**
*Tahltan 2892	N/A	CTAB (1)	yes	PCR contamination
*Tahltan 4758	N/A	CTAB (1)	yes	PCR contamination
Tahltan 5527	N/A	CTAB (3)	yes	3 times DNA extract contaminated with foreign DNA
*Historic Dog 41/42	Rib, ulna radius	Persson (1) Thomas, *et al.* (1)	no yes	No amplification Clean extract, sequenced
Wool Dog 17	calcaneus	Persson (1)	no	No amplification of extract
*Wool Dog 64	rib	Persson (2) Thomas (1)	yes/no yes	1 unique sequence in 1st extract (blank and PCR neg seq'd also) Several identical sequences same as 1st unique seq. (PCR neg seq'd)
Wool Dog 65	rib	Persson (1)	no	PCR contamination, no amplification
Wool Dog 68	tibia	Persson (1)	no	PCR contamination, no amplification
Village Dog 19	humerus	Persson (3)	yes/no/no	1st extraction clean; sequenced, 2nd and 3rd extractions; no amplification
Village Dog 72	rib	Richards & Sykes (1) Persson (1) Thomas, *et al* (1)	yes no yes	DNA and Blank extractions sequenced No amplification of extract Contaminated PCR, dog and PCR neg seq'd; no unique sequences
Village Dog 73	long	Persson (1)	no	No amplification of extract
*Village Dog 74	femur	Persson (2)	yes	Both extracts and blanks sequenced, unique sequences in bone
Prehistoric Dog 30	rib	Persson (1)	no	Contaminated
Prehistoric Dog 31	rib	Persson (1)	yes	PCR contamination, dog and PCR neg seq'd; no unique sequences
VI Wolf 53	ulna	Persson (1)	no	Contaminated
VI Wolf 56	metacarpal	Thomas, *et al* (1)	yes	PCR contamination, wolf, blank and PCR neg seq'd; no unique seqs
Tuna 34	dentary	Persson (1)	no	No amplification
Tuna 35	maxilla	Persson (2)	no	PCR contamination, tuna, blank and PCR neg seq'd; no unique sequences

Table 3.2 Results of the ancient DNA extractions and PCR reactions (other than those performed by S.A.B.). Some of the samples were extracted only once as a result of time constraints;others were extracted by S.A.B. as well.

Sample	Extractions	Clean Extracts	Contaminated Extracts	Failed Extracts	Clean PCRs	Contaminated PCRs	Failed PCRs
Tahltan 2892	1	1	0	0	0	1	1
Tahltan 4758	1	1	0	0	0	1	3
Tahltan 5527	3	1	1	1	1	5	2
Historic Dog 41	2	2	0	0	2	4	6
Wool Dog 17	1	0	0	1	0	0	1
Wool Dog 64	3	1	1	1	1	8	5
Wool Dog 65	1	0	0	1	0	4	3
Wool Dog 68	1	0	0	1	0	2	0
Village Dog 19	3	1	0	2	1	1	5
Village Dog 72	3	1	1	1	2	5	10
Village Dog 73	1	0	1	0	3	1	1
Village Dog 74	2	0	2	0	5	2	3
Prehistoric Dog 30	1	0	1	0	1	2	7
Prehistoric Dog 31	1	0	1	0	1	0	0
Van Isld Wolf 53	1	0	1	0	1	1	3
Van Isld Wolf 56	1	0	1	0	0	3	0
Tuna 34	1	0	0	1	0	1	0
Tuna 35	2	0	0	1	0	0	3

Table 3.3 Key for previously published haplotypes for eight indigenous Japanese dog breeds and several other non Japanese breeds (Okumura *et al.*, 1996). Non Japanese breeds which share a haplotype with a Japanese breed are denoted by an *. Numbers of individuals are in parentheses, and the haplotypes are named according to their GenBank accession number.

	Haplotype	**Breeds**		**Haplotype**	**Breeds**
Japanese	D83600	Akita (2)	**Japanese**	D83630	Shiba (9), Pekingnese* (1)
	D83601	Akita (5)		D83631	Shiba (1)
	D83607	Hokkaido (3), Akita (3), Iki (2)		D83632	Shiba (1)
	D83608	Hokkaido (1)		D83636	Shikoku (1)
	D83609	Hokkaido (3), Shikoku (1), Kishu (1), Shiba (1), Akita (1), Pug* (1)		D83633	Shiba (4), Akita (1), Hokkaido (1), Iki (2), Kai (1), Dalmation* (1), Beagle* (1), Poodle* (1), Eskimo* (1)
	D83610	Iki (3)	**Non Japanese**	D83599	Afghan (1)
	D83611	Kishu (1)		D83602	Cavelier King Charles Spaniel (1)
	D83612	Kishu (1)		D83603	Collie (1)
	D83613	Kishu (1)		D83604	Doberman (1)
	D83618	Ryukyu (4)		D83605	Eskimo (1)
	D83619	Ryukyu (1)		D83606	Great Pyrenees (1)
	D83620	Ryukyu (1), Kishu (1)		D83616	Pointer (1)
	D83621	Ryukyu (2)		D83617	Pug (1)
	D83622	Ryukyu (2)		D83625	Saluki (1)
	D83623	Ryukyu (1)		D83626	Sheltie (2)
	D83624	Ryukyu (1)		D83634	Shi Tzu (1)
	D83627	Shiba (2), Akita (1)		D83635	Shi Tzu (1)
	D83628	Shiba (9), Iki (1)		D83637	Siberian Husky (1)
	D83629	Shiba (1)		D83638	Siberian Husky (1)

Table 3.4 Mean number of transitions (below diagonal) and transversions (above diagonal) between the eight groups revealed through phylogenetic analyses. Letters in brackets correspond to the groups identified in figures 3.3, 3.4 and 3.5.

ti/tv	Fox	Coyote	(A) Wolf	(B) NAm Dog	(C) Wool Dog	(D) Elkhound	(E) Dog	(F) Jpn Dog
Fox	-	35	36.1	36	36	36	36.3	36.5
Coyote	53.5	-	1.1	1	1	1	1.1	1.5
(A) Wolf	61.1	39.1	-	0.18	0.18	0.18	0.56	0.68
(B) NAm Dog	60	37	7.6	-	0	0	0.41	0.5
(C) Wool Dog	59	37.5	11.3	7.5	-	0	0.41	0.5
(D) Elkhound	58	38.5	11.5	12.5	12	-	0.41	0.5
(E) Dog	60.1	36.4	14.4	10.3	10.1	14.5	-	0.91
(F) Jpn Dog	62.5	36	15.7	11	11.5	17.5	9.3	-

Table 3.5 Genetic distance matrix. Distances are averaged between the eight groups revealed through phylogenetic analyses. Letters in brackets correspond to the groups identified in figures 3.3, 3.4 and 3.5.

	Fox	Coyote	(A) Wolf	(B) NAm Dog	(C) Wool Dog	(D) Elkhound	(E) Dog	(F) Jpn Dog
Fox	-							
Coyote	0.15	-						
(A) Wolf	0.17	0.06	-					
(B) NAm Dog	0.16	0.06	0.01	-				
(C) Wool Dog	0.16	0.06	0.02	0.01	-			
(D) Elkhound	0.16	0.06	0.02	0.02	0.02	-		
(E) Dog	0.17	0.06	0.02	0.02	0.02	0.02	-	
(F) Jpn Dog	0.17	0.06	0.02	0.02	0.02	0.03	0.02	-

Fig. 3.1 Multiple alignment of sequence data. The canid sequence data generated by this study are presented in a multiple alignment format (p 65 to 79). Akita 1 is used as a reference sequence, with nucleotides that are identical to akita 1 denoted by dots (.); missing sites, or gaps, are denoted by dashes (-) and polymorphic sites are represented by accepted the one letter notations: Y={CT}, R={AG}, S={CG}, W={AT}, K= {GT}, M={AC}, and N={ACGT}. Several mitochondrial haplotypes were found in more than one individual: greyhound 2 includes greyhound 3, malamute 4, saluki 6 and Siberian husky 9; great Pyrenees 17 is identical to greyhound 26; maremma 32 is identical to maremma 31; Vancouver Island wolf 2 is identical to whippet 14, and Vancouver Island wolf 3 includes Arctic wolf 2 and Yukon wolf 1. Genbank Dog and the Other Dog sequences were both obtained from the GenBank database, accession numbers are U03575 and X97343 respectively.

```
                    [        10         20         30         40         50]
Akita 1              GAAATTCTTC TTAAACTATT CCCTGACACC CC-TACATTC ATATATTGAA
Akita 23             ..G....... .......... .......... .......... ..........
Greyhound 2          ..G....... .......... .......... .......... ..........
Gt Pyrenees 17       ..G....... .......... .......... .......... ..........
Gt Pyrenees 27       ..G.....Y. .......... .......... .......... ..........
Malamute 20          ..G....... .......... .......... .......... ..........
Malamute 22          ..G....... .......... .......... .......... ..........
Malamute 24          ..G.....Y. .......... .......... .......... ..........
Maremma 28           ..G....... .......... .......... .......... ..........
Maremma 31           ..G.....Y. .......... .......... .......... ..........
Maremma 33           ..G.....Y. .......... .......... .......... ..........
Samoyed 7            ..G.....Y. .......... .......... .......... ..........
Samoyed 8            ..G....... .......... .......... .......... ..........
Sib Husky 10         ..G.....Y. .......... .......... .......... ..........
Sib Husky 11         ..G.....Y. .......... .......... .......... ..........
Shar Pei 25          ..G.....Y. .......... .......... .......... ..........
Whippet 13           ..G.....Y. .......... .......... .......... ..........
Whippet 15           ..G....... .......... .......... .......... ..........
Whippet 16           ..G....... .......... .......... .......... ..........
Whippet 18           ..G....... .......... .......... .......... ..........
GenBank Dog          ..G....... .......... .......... .......... ..........
The Other Dog        ---------- ---------- -----..... .......... ..........
Nrw Elkhound 5       .......... .......... .......... .......... ..........
Nrw Elkhound 19      .......... .......... .......... .......... ..........
Arctic Wolf 1        .......... .......... .......... .......... ..........
European Wolf 1      .......... .......... .......... .......... ..........
European Wolf 2      .......... .......... .......... .......... ..........
Yukon Wolf 2         .......... .......... .......... .......... ..........
Yukon Wolf 4         .......... .......... .......... .......... ..........
Yukon Wolf 5         .......... .......... .......... .......... ..........
Yukon Wolf 6         .......... .......... .......... .......... ..........
Yukon Wolf 22        .......... .......... .......... .......... ..........
Van Isld Wolf 1      ..G....... .......... .......... .......... ..........
Van Isld Wolf 2      ..G....... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... .......... ..........
Tahltan 1 (2892)     ---------- ----...... .......... ........Y. ..........
Tahltan 2 (4758)     ---------- ----...... .......... .......... ..........
Historic Dog 41      ---------- ----...... .......... .......... ..........
Village Dog 74       ---------- ----...... .......... .......... ..........
Wool Dog 64          ---------- ----...... .......... .......... ..........
Coyote 14            .......... .......... ......TG.. .......... ..........
Coyote 15            .......... .......... ......TG.. .......... ..........
Coyote 16            .......... .......... ......TG.. ..C....... ..........
Fox                  .........T .......... .......G.. .-.-.....A .C-C.A..TG
```

Fig. 3.1

```
                     [        60         70         80         90        100]
Akita 1              TCACCCCTAC TGTGCTATGT CAGTATCTCC AGGTAAACCC TTCTCCCCTC
Akita 23             .......... .......... .......... .......... ..........
Greyhound 2          .......... .......... .......... .......... ..........
Gt Pyrenees 17       .......... .......... .......... .......... ..........
Gt Pyrenees 27       .......... .......... .......... .......... ..........
Malamute 20          .......... .......... .......... .......Y.. ..........
Malamute 22          .......... .......... .......... .......... ..........
Malamute 24          .......... .......... .......... .......... ..........
Maremma 28           .......... .......... .......... .......... ..........
Maremma 31           .......... .......... .......... .......... ..........
Maremma 33           .......... .......... .......... .......... ..........
Samoyed 7            .......... .......... .......... .......... ..........
Samoyed 8            .......... .......... .......... .......... ..........
Sib Husky 10         .......... .......... .......... .......... ..........
Sib Husky 11         .......... .......... .......... .......... ..........
Shar Pei 25          .......... .......... .......... .......... ..........
Whippet 13           .......... .......... .......... .......... ..........
Whippet 15           .......... .......... .......... .......... ..........
Whippet 16           .......... .......... .......... .......... ........Y.
Whippet 18           .......... .......... .......... .......... ..........
GenBank Dog          .......... .......... .......... .......... ..........
The Other Dog        .......... .......... .......... .......... ..........
Nrw Elkhound 5       .......... .......... .......... .......... ..........
Nrw Elkhound 19      .......... .......... .......... .......... ..........
Arctic Wolf 1        .......... .......... .......... .......... ....T.....
European Wolf 1      .......... .......... .......... .......... ....T.....
European Wolf 2      .......... .......... .......... .......... ....T.....
Yukon Wolf 2         .......... .......... .......... .......... ....T.....
Yukon Wolf 4         .......... .......... .......... .......... ....T.....
Yukon Wolf 5         .......... .......... .......... .......... ....T.....
Yukon Wolf 6         .......... .......... .........Y .......... ....T.....
Yukon Wolf 22        .......... .......... .......... .......... ....T.....
Van Isld Wolf 1      .......... .......... .......... .......... ..........
Van Isld Wolf 2      .......... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... .......... ....T.....
Tahltan 1 (2892)     .......... .......... .......... .......... ....T.....
Tahltan 2 (4758)     .......... .......... .......... .......... ....T.....
Historic Dog 41      .......... .......... .......... .......... ....T.....
Village Dog 74       .......... .......... .......... .......... ....T.....
Wool Dog 64          .......... .......... ......T... .......... ....T.....
Coyote 14            .......... .......C.. .......... .A--...T.. ..........
Coyote 15            .......... .......C.. .......... .A--...T.. ..........
Coyote 16            .......... .......C.. .......... .A--...T.. ..........
Fox                  .T..G---.. .A.C.CGC.C T------C.. .---...--. ..--G...--
```

Fig. 3.1 (Continued)

```
                    [        110        120        130        140        150]
Akita 1              CCC-TATGTA CGTCGTGCAT TAATGGTTTG CCCCATGCAT AT-AAGCATG
Akita 23             .......... .......... .......... .......... ..........
Greyhound 2          .......... .......... .......... .......... ..........
Gt Pyrenees 17       .......... .......... .......... .......... ..........
Gt Pyrenees 27       .......... .......... .......... .......... ..........
Malamute 20          .......... .......... ......C... .......... ..........
Malamute 22          .......... .......... ......C... .......... ..........
Malamute 24          .......... .......... .......... .......... ..........
Maremma 28           .......... .......... .......... .......... ..........
Maremma 31           .......... .......... .......... .......... ..........
Maremma 33           .......... .......... .......... .......... ..........
Samoyed 7            .......... .......... .......... .......... ..........
Samoyed 8            .......... .......... .......... .......... ..........
Sib Husky 10         .......... .......... .......... .......... ..........
Sib Husky 11         .......... .......... .......... .......... ..........
Shar Pei 25          .......... .......... .......... .......... ..........
Whippet 13           .......... .......... .......... .......... ..........
Whippet 15           .......... .......... .......... .......... ..........
Whippet 16           .......... .......... .......... .......... ..........
Whippet 18           .......... .......... .......... .......... ..........
GenBank Dog          .......... .......... .......... .......... ..........
The Other Dog        .......... .......... ..R....... .......... ..........
Nrw Elkhound 5       .......... .......... .......... .......... ..........
Nrw Elkhound 19      .......... .......... .......... .......... ..........
Arctic Wolf 1        .......... .......... .......... .......... ..........
European Wolf 1      .......... .......... .......... .......... ..........
European Wolf 2      .......... .......... .......... .......... ..........
Yukon Wolf 2         .......... .......... .......... .......... ..........
Yukon Wolf 4         ...C...... .......... .......... .......... ..........
Yukon Wolf 5         .......... .......... .......... .......... ..........
Yukon Wolf 6         .......... .......... .......... ........R. ..........
Yukon Wolf 22        ...C...... .......... .......... .......... ..........
Van Isld Wolf 1      .......... .......... .......... .......... ..........
Van Isld Wolf 2      .......... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... .......... ..........
Tahltan 1 (2892)     ...C...... .......... .......... .......... ..........
Tahltan 2 (4758)     ...C...... .......... .......... .......... ..........
Historic Dog 41      ...C...... .......... .......... .......... ..........
Village Dog 74       ...C...... .......... .......... .......... ..........
Wool Dog 64          .......... .......... .......... .......... ..........
Coyote 14            -......... .......... ......C... .......... ..........
Coyote 15            -......... .......... ......C... .......... ..........
Coyote 16            -......... .......... ......C... .......... ..........
Fox                  ---....... .......... ..C...CA.. .......... ..T.......
```

Fig. 3.1 (Continued)

```
                    [        160        170        180        190        200]
Akita 1             TACATAATAT TATATCCTTA CATAGGACAT ATTA-ACTCA ATCTCATAGT
Akita 23            .......... .......... .......... .......... .C........
Greyhound 2         .......... .......... .......... .......... ........A.
Gt Pyrenees 17      .......... .......... .......... .......... ..........
Gt Pyrenees 27      .......... .......... .......... .......... ..........
Malamute 4          .......... .......... .......... .......... ........A.
Malamute 22         .......... .......... .......... .......... ........A.
Malamute 24         .......... .......... .......... .......... .C........
Maremma 28          .......... .......... .......... .......... ........A.
Maremma 31          .......... .......... .......... .......... ........A.
Maremma 33          .......... .......... .......... .......... ........A.
Samoyed 7           .......... .......... .......... .......... .C........
Samoyed 8           .......... .......... .......... .......... .C........
Sib Husky 10        .......... .......... .......... .......... ........A.
Sib Husky 11        .......... .......... .......... .......... ..........
Shar Pei 25         .......... .......... .......... .......... ..........
Whippet 13          .......... .......... .......... .......... ........A.
Whippet 15          .......... .......... .......... .......... ........A.
Whippet 16          .......... .......... .......... .......... ........A.
Whippet 18          .......... .......... .......... .......... ........A.
GenBank Dog         .......... .......... .......... .......... ..........
The Other Dog       .......... .......... .......... .......... ........R.
Nrw Elkhound 5      .......... .......... .......... .......... ......C.A.
Nrw Elkhound 19     .......... .......... .......... .......... ......C.A.
Arctic Wolf 1       .......... .....T.... .......... ..C....... ........A.
European Wolf 1     .......... .....T.... .......... ..C....... ........A.
European Wolf 2     .......... .....T.... .......... ..C....... ......C.A.
Yukon Wolf 2        .......... .....T.... .......... ...G...... ...C..C.A.
Yukon Wolf 4        .......... .....T.... .......... .......... ........A.
Yukon Wolf 5        .......... .....T.... .......... ..C....... ......C.A.
Yukon Wolf 6        .......... .....T.... .......... ..C....... ......C.A.
Yukon Wolf 22       .......... .....T.... .......... .......... ..T.....A.
Van Isld Wolf 1     .......... .......... .......... .......... ........A.
Van Isld Wolf 2     .......... .......... .......... .......... ........A.
Van Isld Wolf 3     .......... .....T.... .......... ..C....... ......C.A.
Tahltan 1 (2892)    .......... .....T.... .......... R......... ..T.....A.
Tahltan 2 (4758)    .......... .....T.... .......... .......... ..T.....A.
Historic Dog 41     .......... .....T.... .......... ........Y. ..T.....A.
Village Dog 74      .......... .....T.... .......... .......... ..T.....A.
Wool Dog 64         .......... .......... .......... .C........ ........A.
Coyote 14           .......... .......... .......... ...G...T.. .C..T.C...
Coyote 15           .......... .......... .......... ...G....T. .C..T.C...
Coyote 16           .......... .......... .......... .C.G....T. .C..T.C...
Fox                 ......TAC. .....AA... .....A.... .C..TGT.T. .T..T.C.A.
```

Fig. 3.1 (Continued)

```
                    [        210        220        230        240        250]
Akita 1             TCACTGATCT ATCAACAGT- -AATCGAATG CATATCACTT AGTCCAATAA
Akita 23            .......... .......... .......... ........C. ..........
Greyhound 2         .......... .......... .......... .......... ..........
Gt Pyrenees 17      .......... G......... .......... .......... ..........
Gt Pyrenees 27      ...T...... .......... .......... .......... ..........
Malamute 20         .......... .......... .......... .......... ..........
Malamute 22         .......... .......... .......... .......... ..........
Malamute 24         .......... .......... .......... .......... ..........
Maremma 28          .......... .......... .......... .......... ..........
Maremma 31          .......... .......... .......... .......... ..........
Maremma 33          .......... W......... .......... .......... ..........
Samoyed 7           .......... .......... .......... .......... ..........
Samoyed 8           .......... .......... .......... .......... ..........
Sib Husky 10        .......... .......... .......... .......... ..........
Sib Husky 11        .......... .......... .......... .......... ..........
Shar Pei 25         .......... .C........ .....A.... ........C. ..........
Whippet 13          .......... T......... .......... .......... ..........
Whippet 15          .......... T......... .......... .......... ..........
Whippet 16          .......... T......... .......... .......... ..........
Whippet 18          .......... T......... .......... .......... ..........
GenBank Dog         .....C.... .......... .....A.... .......... ..........
The Other Dog       .....S.... .......... .....R.... .......... ..........
Nrw Elkhound 5      ...T.A.C.. G...G..... .......... .......... ..........
Nrw Elkhound 19     ...T.A.C.. G...G..... .......... .......... ..........
Arctic Wolf 1       ...T...... R......... .....A.... .......... ..........
European Wolf 1     ...T...... G......... .....A.... .......... ..........
European Wolf 2     ...T...... .......... .....A.... .......... ..........
Yukon Wolf 2        ...T...... .......... .....A.... ..C....... ..........
Yukon Wolf 4        .......... ....G..... .....A.... .......... ..........
Yukon Wolf 5        ...T...... ....G..... .....A.... .......... ..........
Yukon Wolf 6        ...T...... ....G..... .....A.... .......... ..........
Yukon Wolf 22       .......... .......... .....A.... .......... ..........
Van Isld Wolf 1     .......... T......... .....A.... .......... ..........
Van Isld Wolf 2     .......... T......... .....A.... .......... ..........
Van Isld Wolf 3     ...T...... .......... .....A.... .......... ..........
Tahltan 1 (2892)    .......... .......... .....A.... .......... ..........
Tahltan 2 (4758)    .......... .......... .....R.... .......... ..........
Historic Dog 41     .......... .......... .......... .......... ..........
Village Dog 74      .......... .......... .....A.... ..C....... ..........
Wool Dog 64         .......... G......... ...C...... .......... ....T.....
Coyote 14           C..T...... -......... TGG.TA.... ........C. ..........
Coyote 15           C..T...... -......... TGG.TA.... ........C. ..........
Coyote 16           .......... -......... TGG.TA.... ........C. ..........
Fox                 A-.TC--... ....GG..CA TACAT..C.. ..CG...... ..........
```

Fig. 3.1 (Continued)

```
                    [        260        270        280        290        300]
Akita 1              GGGCTTAATC ACCATGCCTC GAGAAACCAT CAACCCTTGC TCGTAATGTC
Akita 23             .......... .......... .......... .......... ..........
Greyhound 2          .......... .......... .......... .......... ..........
Gt Pyrenees 17       .......... .......... .......... .......... ..........
Gt Pyrenees 27       .......... .......... .......... .......... ..........
Malamute 20          .......... .......... .......... .......... ..........
Malamute 22          .......... .......... .......... .......... ..........
Malamute 24          .......... .......... .......... .......... ..........
Maremma 28           .......... .......... .......... .......... ..........
Maremma 31           .......... .......... .......... .......... ..........
Maremma 33           .......... .......... .......... .......... ..........
Samoyed 7            .......... .......... .......... .......... ..........
Samoyed 8            .......... .......... .......... .......... ..........
Sib Husky 10         .......... .......... .......... .......... ..........
Sib Husky 11         .......... .......... .......... .......... ..........
Shar Pei 25          .......... .......... .......... .......... ..........
Whippet 13           .......... .......... .......... .......... ..........
Whippet 15           .......... .......... .......... .......... ..........
Whippet 16           .......... .......... .......... .......... ..........
Whippet 18           .......... .......... .......... .......... ..........
GenBank Dog          .......... .......... .......... .......... ..........
The Other Dog        .......... .......... .......... .......... ..........
Nrw Elkhound 5       .......... .......... .......... .......... ..........
Nrw Elkhound 19      .......... .......... .......... .......... ..........
Arctic Wolf 1        .......... .......... .......... .......... ..........
European Wolf 1      .......... .......... .......... .......... ..........
European Wolf 2      .......... .......... .......... .......... ..........
Yukon Wolf 2         .......... .......... .......... .......... ..........
Yukon Wolf 4         .......... .......... .......... .......... ..........
Yukon Wolf 5         .......... .......... .......... .......... ..........
Yukon Wolf 6         .......... .......... .......... .......... ..........
Yukon Wolf 22        .......... .......... .......... .......... ..........
Van Isld Wolf 1      .......... .......... .......... .......... ..........
Van Isld Wolf 2      .......... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... .......... ..........
Tahltan 1 (2892)     .......... .......... .......... .......... ..........
Tahltan 2 (4758)     .......... .......... .......... .......... ..........
Historic Dog 41      .......... .......... .......... ........C. ..........
Village Dog 74       .......... .......... .......... .......... ..........
Wool Dog 64          .......... .......... .......... .......... ..........
Coyote 14            .......... .......... .......... ...T...... ..........
Coyote 15            .......... .......... .......... ...T...... ..........
Coyote 16            .......... .......... .......... ...T...... ..........
Fox                  ...A..T... .......... .......... ...T...... ...A.G.A..
```

Fig. 3.1 (Continued)

```
                    [       300        310        320        330        350]
Akita 1              CCTCTTCTCG CTCCGGGCCC ATACCAACGT GGGGGTTACT ATCATGAAAC
Akita 23             .......... .......... ....T..... .......... ..........
Greyhound 2          .......... .......... ....T..... .......... ..........
Gt Pyrenees 17       .......... .......... ....T..... .......... ..........
Gt Pyrenees 27       .......... .......... ....T..... .......... ..........
Malamute 20          .......... .......... ....T..... .......... ..........
Malamute 22          .......... .......... ....T..... .......... ..........
Malamute 24          .......... .......... ....T..... .......... ..........
Maremma 28           .......... .......... ....T..... .......... ..........
Maremma 31           .......... .......... ....T..... .......... ..........
Maremma 33           .......... .......... ....T..... .......... ..........
Samoyed 7            .......... .......... ....T..... .......... ..........
Samoyed 8            .......... .......... ....T..... .......... ..........
Sib Husky 10         .......... .......... ....T..... .......... ..........
Sib Husky 11         .......... .......... ....T..... .......... ..........
Shar Pei 25          .......... .......... ....T..... .......... ..........
Whippet 13           .......... .......... ....T..... .......... ..........
Whippet 15           .......... .......... ....T..... .......... ..........
Whippet 16           .......... .......... ....T..... .......... ..........
Whippet 18           .......... .......... ....T..... .......... ..........
GenBank Dog          .......... .......... ....T..... .......... ..........
The Other Dog        .......... .......... ....T..... .......... ........-.
Nrw Elkhound 5       .......... .......... ....T..... .......... .....-----
Nrw Elkhound 19      .......... .......... ....T..... .......... ..........
Arctic Wolf 1        .......... .......... ....T..... .......... ..........
European Wolf 1      .......... .......... ....T..... .......... ..........
European Wolf 2      .......... .......... ....T..... .......... ..........
Yukon Wolf 2         .......... .......... ....T..... .......... ......G...
Yukon Wolf 4         .......... .......... ....T..... .......... ..........
Yukon Wolf 5         .......... .......... ....T..... .......... ..........
Yukon Wolf 6         .......... .......... ....T..... .......... ..........
Yukon Wolf 22        .......... .......... ....T..... .......... ..........
Van Isld Wolf 1      .......... .......... ....T..... .......... ..........
Van Isld Wolf 2      .......... .......... ....T..... .......... ..........
Van Isld Wolf 3      .......... .......... ....T..... .......... ..........
Tahltan 1 (2892)     .......... ......-..- ---------- ---------- ----------
Tahltan 2 (4758)     .......... .........- ---------- ---------- ----------
Historic Dog 41      .......... .........- ---------- ---------- ----------
Village Dog 74       .......... .........- ---------- ---------- ----------
Wool Dog 64          .......... .........- ---------- ---------- ----------
Coyote 14            .......... .......... ...T...... .......T.. ..T...G...
Coyote 15            .......... .......... ...T...... .......T.. ..T...G...
Coyote 16            .......... .......... ...T...... .......T.. ......G...
Fox                  .......... .......... ...T...... .......T.. ......G...
```

Fig. 3.1 (Continued)

```
                      [        360        370        380        390       400]
Akita 1                TATACCTGGC ATCTGGTTCT TACTTCAGGG CCATAAC-TT TATTTACTCC
Akita 23               .......... .......... .......... .......... ..........
Greyhound 2            .......... .......... .......... .......... ..........
Gt Pyrenees 17         .......... .......... .......... .......... ..........
Gt Pyrenees 27         .......... .......... .......... .......... ..........
Malamute 20            .......... .......... .......... .......... ..........
Malamute 22            .......... .......... .......... .......... ..........
Malamute 24            .......... .......... .......... .......... ..........
Maremma 28             .......... .......... .......... .......... ..........
Maremma 31             .......... .......... .......... .......... ..........
Maremma 33             .......... .......... .......... .......... ..........
Samoyed 7              .......... .......... .......... .......... ..........
Samoyed 8              .......... .......... .......... .......... ..........
Sib Husky 10           .......... .......... .......... .......... ..........
Sib Husky 11           .......... .......... .......... .......... ..........
Shar Pei 25            .......... .......... .......... .......... ..........
Whippet 13             .......... .......... .......... .......... ..........
Whippet 15             .......... .......... .......... .......... ..........
Whippet 16             .......... .......... .......... .......... ..........
Whippet 18             .......... .......... .......... .......... ..........
GenBank Dog            ---------- ---------- ---------- ---------- ----------
The Other Dog          .......... .......... .........C .......... ..........
Nrw Elkhound 5         .......... .......... ...C...... .........C ..........
Nrw Elkhound 19        .......... .......... ...C...... .........C ..........
Arctic Wolf 1          .......... .......... ...C...... .......... ..........
European Wolf 1        .......... .......... ...C...... .......... ..........
European Wolf 2        .......... .......... ...C...... .......... ..........
Yukon Wolf 2           .......... .......... ...C...... .......... ..........
Yukon Wolf 4           .......... .......... ...C...... .......... ..........
Yukon Wolf 5           .......... .......... ...C...... .......... ..........
Yukon Wolf 6           .......... .......... ...C...... .......... ..........
Yukon Wolf 22          .......... .......... ...C...... .......... ..........
Van Isld Wolf 1        .......... .......... .......... ........C. ..........
Van Isld Wolf 2        .......... .......... .......... ........C. ..........
Van Isld Wolf 3        .......... .......... ...C...... .......... ..........
Tahltan 1 (2892)       ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)       ---------- ---------- ---------- ---------- ----------
Historic Dog 41        ---------- ---------- ---------- ---------- ----------
Village Dog 74         ---------- ---------- ---------- ---------- ----------
Wool Dog 64            ---------- ---------- ---------- ---------- ----------
Coyote 14              .......... .......... ...C...... ....G.T.C. ..........
Coyote 15              .......... .......... ...C...... ....G.T.C. ..........
Coyote 16              .......... .......... ...C...... ......T.C. ..........
Fox                    .......... .......... ...C...... ....TCTGC. .G..C.....
```

Fig. 3.1 (Continued)

```
                     [        410        420        430        440        450]
Akita 1              AATCCTACTA ATTCTCGCAA ATGGGACATC TCGATGGACT AATGACTAAT
Akita 23             .......... .......... .......... .......... ..........
Greyhound 2          .......... .......... .......... .......... ..........
Gt Pyrenees 17       .......... .......... .......... .......... ..........
Gt Pyrenees 27       .......... .......... .......... .......... ..........
Malamute 20          .......... .......... .......... .......... ..........
Malamute 22          .......... .......... .......... .......... ..........
Malamute 24          .......... .......... .......... .......... ..........
Maremma 28           .......... .......... .......... .......... ..........
Maremma 31           .......... .......... .......... .......... ..........
Maremma 33           .......... .......... .......... .......... ..........
Samoyed 7            .......... .......... .......... .......... ..........
Samoyed 8            .......... .......... .......... .......... ..........
Sib Husky 10         .......... .......... .......... .......... ..........
Sib Husky 11         .......... .......... .......... .......... ..........
Shar Pei 25          .......... .......... .......... .......... ..........
Whippet 13           .......... .......... .......... .......... ..........
Whippet 15           .......... .......... .......... .......... ..........
Whippet 16           .......... .......... .......... .......... ..........
Whippet 18           .......... .......... .......... .......... ..........
GenBank Dog          ---------- ---------- ---------- ---------- ----------
The Other Dog        .......... .......... .......... .......... ..........
Nrw Elkhound 5       .......... .......... ..A....... .......... ..........
Nrw Elkhound 19      .......... .......... ..A....... .......... ..........
Arctic Wolf 1        .......... .......... .......... .......... ..........
European Wolf 1      .......... .......... .......... .......... ..........
European Wolf 2      .......... .......... .......... .......... ..........
Yukon Wolf 2         .......... .......... .......... .......... ..........
Yukon Wolf 4         .......... .......... .......... .......... ..........
Yukon Wolf 5         .......... .......... .......... .......... ..........
Yukon Wolf 6         .......... .......... .......... .......... ..........
Yukon Wolf 22        .......... .......... .......... .......... ..........
Van Isld Wolf 1      .......... .......... .......... .......... ..........
Van Isld Wolf 2      .......... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... .......... ..........
Tahltan 1 (2892)     ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)     ---------- ---------- ---------- ---------- ----------
Historic Dog 41      ---------- ---------- ---------- ---------- ----------
Village Dog 74       ---------- ---------- ---------- ---------- ----------
Wool Dog 64          ---------- ---------- ---------- ---------- ----------
Coyote 14            .......... .....T.... .......... .......... ..........
Coyote 15            .......... .....T.... .......... .......... ..........
Coyote 16            .......... .....T.... .......... .......... ..........
Fox                  .......... ..C...T... .......... .......... ..........
```

Fig. 3.1 (Continued)

```
                     [        460        470        480        490       500]
Akita 1              CAGCCCATGA TCACACATAA CTGTGGTGTC ATGCATCTGG TATCTTTTAA
Akita 23             .......... .......... .......... .......... ..........
Greyhound 2          .......... .......... .......... .......... ..........
Gt Pyrenees 17       .......... .......... .......... .......... ..........
Gt Pyrenees 27       .......... .......... .......... .......... ..........
Malamute 20          .......... .......... .......... .......... ..........
Malamute 22          .......... .......... .......... .......... ..........
Malamute 24          .......... .......... .......... .......... ..........
Maremma 28           .......... .......... .......... .......... .........T
Maremma 31           .......... .......... .......... .......... .........T
Maremma 33           .......... .......... .......... .......... ..........
Samoyed 7            .......... .......... .......... .......... ..........
Samoyed 8            .......... .......... .......... .......... ..........
Sib Husky 10         .......... .......... .......... .......... ..........
Sib Husky 11         .......... .......... .......... .......... ..........
Shar Pei 25          .......... .......... .......... .......... ..........
Whippet 13           .......... .......... .......... .......... ..........
Whippet 15           .......... .......... .......... .......... ..........
Whippet 16           .......... .......... .......... .......... ..........
Whippet 18           .......... .......... .......... .......... ..........
GenBank Dog          ---------- ---------- ---------- ---------- ----------
The Other Dog        .......... .......... ....K..... ......Y... ..........
Nrw Elkhound 5       .......... .......... .......... ......T... ..........
Nrw Elkhound 19      .......... .......... .......... ......T... ..........
Arctic Wolf 1        .......... .......... .......... ......T... ..........
European Wolf 1      .......... .......... .......... ......T... ..........
European Wolf 2      .......... .......... .......... ......T... ..........
Yukon Wolf 2         .......... .......... .......... ......T... ..........
Yukon Wolf 4         .......... .......... .......... ......T... ..........
Yukon Wolf 5         .......... .......... .......... ......T... ..........
Yukon Wolf 6         .......... .......... .......... ......T... ..........
Yukon Wolf 22        .......... .......... .......... ......T... ..........
Van Isld Wolf 1      .......... .......... .......... .......... ..........
Van Isld Wolf 2      .......... .......... .......... .......... ..........
Van Isld Wolf 3      .......... .......... .......... ......T... ..........
Tahltan 1 (2892)     ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)     ---------- ---------- ---------- ---------- ----------
Historic Dog 41      ---------- ---------- ---------- ---------- ----------
Village Dog 74       ---------- ---------- ---------- ---------- ----------
Wool Dog 64          ---------- ---------- ---------- ---------- ----------
Coyote 14            .......... .......... .......... ......T... ..........
Coyote 15            .......... .......... .......... ......T... ..........
Coyote 16            .......... .......... .......... ......T... ..........
Fox                  .......... .......... .......... ......T... .....C..-T
```

Fig. 3.1 (Continued)

```
                     [         510        520        530        540        550]
Akita 1               TTTTT-AGGG GGG-AATCTG CTATCACTCA CCTACGACCG CAACGGCACT
Akita 23              .......... ...G...... .......... .......... ..........
Greyhound 2           .......... ...G...... .......... .......... ..........
Gt Pyrenees 17        .......... ...G...... .......... .......... ..........
Gt Pyrenees 27        .......... ...G...... .......... .......... ..........
Malamute 20           .......... ...G...... .......... .......... ..........
Malamute 22           .......... ...G...... .......... .......... ..........
Malamute 24           .......... ...G...... .......... T......... ..........
Maremma 28            .......... ...G...... .......... .......... ..........
Maremma 31            .......... ...G...... .......... .......... ..........
Maremma 33            .......... ...G...... .......... .......... ..........
Samoyed 7             .......... ...G...... .......... .......... ..........
Samoyed 8             .......... ...G...... .......... .......... ..........
Sib Husky 10          .......... ...G...... .......... .......... ..........
Sib Husky 11          ......-... ...G...... .......... T......... ..........
Shar Pei 25           .......... ...G...... .......... .......... ..........
Whippet 13            .......... ...G...... .......... .......... ..........
Whippet 15            .......... ...G...... .......... .......... ..........
Whippet 16            .......... ...G...... .......... .......... ..........
Whippet 18            .......... ...G...... .......... .......... ..........
GenBank Dog           ---------- ---------- ---------- ---------- ----------
The Other Dog         .......... ...G...... .......... .M........ .M........
Nrw Elkhound 5        .......... ...G...... .......... T...T..... ..........
Nrw Elkhound 19       .......... ...G...... .......... T...T..... ..........
Arctic Wolf 1         .......... ...G...... .......... T......... ..........
European Wolf 1       .......... .......... .......... T......... ..........
European Wolf 2        .......... .......... .......... T......... ..........
Yukon Wolf 2          .......... .......... .......... T......... ..........
Yukon Wolf 4          .......... ...G...... .......... T......... ..........
Yukon Wolf 5          .......... .......... .......... T......... ..........
Yukon Wolf 6          .......... .......... .......... T......... ..........
Yukon Wolf 22         .......... ...G...... .......... T......... ..........
Van Isld Wolf 1       .......... ...G...... .......... .......... ..........
Van Isld Wolf 2       .......... ...G...... .......... .......... ..........
Van Isld Wolf 3       .......... ...G...... .......... T......... ..........
Tahltan 1 (2892)      ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)      ---------- ---------- ---------- ---------- ----------
Historic Dog 41       ---------- ---------- ---------- ---------- ----------
Village Dog 74        ---------- ---------- ---------- ---------- ----------
Wool Dog 64           ---------- ---------- ---------- ---------- ----------
Coyote 14             ....C.-... ...G...... .......... T......... ..........
Coyote 15             ....C.-... ...G...... .......... T......... ..........
Coyote 16             ....C.-... ...G...... .......... T...T..... ..........
Fox                   .....G.... ...G..CT.. .......... G...T..... ..........
```

Fig. 3.1 (Continued)

```
                     [        560        570        580        590        600]
Akita 1               AACTCTAACT TATCTTCTGC TCTCAGGGAA TATGCCCGTC GCGGCCCTAA
Akita 23              .......... .......... .......... .......... ..........
Greyhound 2           .......... .......... .......... .......... ..........
Gt Pyrenees 17        .......... .......... .......... .......... ..........
Gt Pyrenees 27        .......... .......... .......... .......... ..........
Malamute 20           .......... .......... .......... .......... ..........
Malamute 22           .......... .......... .......... .......... ..........
Malamute 24           .......... .......... .......... .......... ..........
Maremma 28            .......... .......... .......... .......... ..........
Maremma 31            .......... .......... .......... .......... ..........
Maremma 33            .......... .......... .......... .......... ..........
Samoyed 7             .......... .......... .......... .......... ..........
Samoyed 8             .......... .......... .......... .......... ..........
Sib Husky 10          .......... .......... .......... .......... ..........
Sib Husky 11          .......... .......... .......... .......... ..........
Shar Pei 25           .......... .......... .......... .......... ..........
Whippet 13            .......... .......... .......... .......... ..........
Whippet 15            .......... .......... .......... .......... ..........
Whippet 16            .......... .......... .......... .......... ..........
Whippet 18            .......... .......... .......... .......... ..........
GenBank Dog           ---------- ---------- ---------- ---------- ----------
The Other Dog         .......... .......... .......... .......... ..........
Nrw Elkhound 5        .......... .......... ........G. .......... ..........
Nrw Elkhound 19       .......... .......... ........G. .......... ..........
Arctic Wolf 1         .......... .......... .......... .......... ...C......
European Wolf 1       .......... .......... .......... .......... ..........
European Wolf 2       .......... .......... ........G. .......... ..........
Yukon Wolf 2          .......... .......... ........G. .......... ..........
Yukon Wolf 4          .......... .......... ........G. .......... ..........
Yukon Wolf 5          .......... .......... ........G. .......... ..........
Yukon Wolf 6          .......... .......... ........G. .......... ..........
Yukon Wolf 22         .......... .......... ........G. .......... ..........
Van Isld Wolf 1       .......... .......... ........G. .......... ..........
Van Isld Wolf 2       .......... .......... .......... .......... ..........
Van Isld Wolf 3       .......... .......... ........G. .......... ..........
Tahltan 1 (2892)      ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)      ---------- ---------- ---------- ---------- ----------
Historic Dog 41       ---------- ---------- ---------- ---------- ----------
Village Dog 74        ---------- ---------- ---------- ---------- ----------
Wool Dog 64           ---------- ---------- ---------- ---------- ----------
Coyote 14             .......... .......... .......... .......... ..........
Coyote 15             .......... .......... .......... .......... ..........
Coyote 16             .......... .......... .......... .......... ..........
Fox                   .........C ..-.R..... A......... .......... .......CG.
```

Fig. 3.1 (Continued)

```
                    [         610        620        630        640        650]
Akita 1             CGCAGTCAAA TAACTTGTAG CTGGACTTAT TCATTATCAT TTATCAACTC
Akita 23            .......... .......... .......... .......... ..........
Greyhound 2         .......... .......... .......... .......... ..........
Gt Pyrenees 17      .......... .......... .......... .......... ..........
Gt Pyrenees 27      .......... .......... .......... .......... ..........
Malamute 20         T......... .......... .......... .......... ..........
Malamute 22         T......... .......... .......... .......... ..........
Malamute 24         T......... .......... .......... .......... ..........
Maremma 28          .......... .......... .......... .......... ..........
Maremma 31          .......... .......... .......... .......... ..........
Maremma 33          T......... .......... .......... .......... ..........
Samoyed 7           T......... .......... .......... .......... ..........
Samoyed 8           T......... .......... .......... .......... ..........
Sib Husky 10        .......... .......... .......... .......... ..........
Sib Husky 11        .......G.. .......... .......... .......... ..........
Shar Pei 25         T......... .......... .......... .......... ..........
Whippet 13          T......... .......... .......... .......... ..........
Whippet 15          T......... .......... .......... .......... ..........
Whippet 16          T......... .......... .......... .......... ..........
Whippet 18          T......... .......... .......... .......... ..........
GenBank Dog         ---------- ---------- ---------- ---------- ----------
The Other Dog       Y......... .......... .......... .......... ..........
Nrw Elkhound 5      T......... .......... .......... .......... ..........
Nrw Elkhound 19     T......... .......... .......... .......... ..........
Arctic Wolf 1       T......... .......... .......... .......... ..........
European Wolf 1     T......... .......... .......... .......... ..........
European Wolf 2     T......... .......... .......... .......... ..........
Yukon Wolf 2        T......... .......... .......... .......... ..........
Yukon Wolf 4        T......... .......... .......... .......... ..........
Yukon Wolf 5        T......... .......... .......... .......... ..........
Yukon Wolf 6        T......... .......... .......... .......... ..........
Yukon Wolf 22       T......... .......... .......... .......... ..........
Van Isld Wolf 1     T......... .......... .......... .......... ..........
Van Isld Wolf 2     T......... .......... .......... .......... ..........
Van Isld Wolf 3     T......... .......... .......... .......... ..........
Tahltan 1 (2892)    ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)    ---------- ---------- ---------- ---------- ----------
Historic Dog 41     ---------- ---------- ---------- ---------- ----------
Village Dog 74      ---------- ---------- ---------- ---------- ----------
Wool Dog 64         ---------- ---------- ---------- ---------- ----------
Coyote 14           T......... .......... .......... .......... ..........
Coyote 15           T......... .......... .......... .......... ..........
Coyote 16           T......... .......... .......... .......... ..........
Fox                 ........G. .G.TC..... .......... .......... ..........
```

Fig. 3.1 (Continued)

```
                    [         660        670        680        690        700]
Akita 1             ACGCATAAAA -TCAAGGTGC TATTCAGTCA ATGGTTTCAG GACATATAGT
Akita 23            .......... .......... .......... .......... ..........
Greyhound 2         .......... .......... .......... .......... ..........
Gt Pyrenees 17      .......... .......... .......... .......... ..........
Gt Pyrenees 27      .......... .......... .......... .......... ..........
Malamute 20         .......... .......... .......... .......... ..........
Malamute 22         .......... .......... .......... .......... ..........
Malamute 24         .......... .......... .......... .......... ..........
Maremma 28          .......... .......... .......... .......... ..........
Maremma 31          .......... .......... .......... .......... ..........
Maremma 33          .......... .......... .......... .......... ..........
Samoyed 7           .......... .......... .......... .......... ..........
Samoyed 8           .......... .......... .......... .......... ..........
Sib Husky 10        .......... .......... .......... .......... ..........
Sib Husky 11        .......... .......... .......... .......... ..........
Shar Pei 25         .......... .......... .......... .......... ..........
Whippet 13          .......... .......... .......... .......... ..........
Whippet 15          .......... .......... .......... .......... ..........
Whippet 16          .......... .......... .......... .......... ..........
Whippet 18          .......... .......... .......... .......... ..........
GenBank Dog         ---------- ---------- ---------- ---------- ----------
The Other Dog       .......... .......... .......... .......... ..........
Nrw Elkhound 5      .......... .......... .......... .......... ..........
Nrw Elkhound 19     .......... .......... .......... .......... ..........
Arctic Wolf 1       ........G. .......... .......... .......... ..........
European Wolf 1     ........G. .......... .......... .......... ..........
European Wolf 2     ........G. .......... .......... .......... ..........
Yukon Wolf 2        ........G. .......... .......... .......... ..........
Yukon Wolf 4        .......... .......... .......... .......... ..........
Yukon Wolf 5        ........G. .......... .......... .......... ..........
Yukon Wolf 6        ........G. .......... .......... .......... ..........
Yukon Wolf 22       .......... .......... .......... .......... ..........
Van Isld Wolf 1     .......... .......... .......... .......... ..........
Van Isld Wolf 2     .......... .......... .......... .......... ..........
Van Isld Wolf 3     ........G. .......... .......... .......... ..........
Tahltan 1 (2892)    ---------- ---------- ---------- ---------- ----------
Tahltan 2 (4758)    ---------- ---------- ---------- ---------- ----------
Historic Dog 41     ---------- ---------- ---------- ---------- ----------
Village Dog 74      ---------- ---------- ---------- ---------- ----------
Wool Dog 64         ---------- ---------- ---------- ---------- ----------
Coyote 14           .......... .......... .......... .......... ........A.
Coyote 15           .......... .......... .......... .......... ........A.
Coyote 16           .......... .......... .......... .......... ........A.
Fox                 -..TGC.C.. C......... .......... .......... ......AG.A
```

Fig. 3.1 (Continued)

```
                      [       710       718]
Akita 1               TTT-A----- GGGTACAC
Akita 23              .......... ........
Greyhound 2           .......... ........
Gt Pyrenees 17        .......... ........
Gt Pyrenees 27        .......... ........
Malamute 20           .......... ........
Malamute 22           .......... ........
Malamute 24           .......... ........
Maremma 28            .......... ........
Maremma 31            .......... ........
Maremma 33            .......... ........
Samoyed 7             .......... ........
Samoyed 8             .......... ........
Sib Husky 10          .......... ........
Sib Husky 11          .......... ........
Shar Pei 25           .......... ........
Whippet 13            .......... ........
Whippet 15            .......... ........
Whippet 16            .......... ........
Whippet 18            .......... ........
GenBank Dog           ---------- --------
The Other Dog         .......... ...-....
Nrw Elkhound 5        .......... ........
Nrw Elkhound 19       .......... ........
Arctic Wolf 1         .......... ........
European Wolf 1       .......... ........
European Wolf 2       .......... ........
Yukon Wolf 2          .......... ........
Yukon Wolf 4          .......... ........
Yukon Wolf 5          .......... ........
Yukon Wolf 6          .......... ........
Yukon Wolf 22         .......... ........
Van Isld Wolf 1       .......... ........
Van Isld Wolf 2       .......... ........
Van Isld Wolf 3       .......... ........
Tahltan 1 (2892)      ---------- --------
Tahltan 2 (4758)      ---------- --------
Historic Dog 41       ---------- --------
Village Dog 74        ---------- --------
Wool Dog 64           ---------- --------
Coyote 14             .C........ ..AC....
Coyote 15             .C........ ..AC....
Coyote 16             .C........ ..AC....
Fox                   A..T.CACAC .TAC....
```

Fig. 3.1 (Continued)

GAGATTCTYC TTAAACTATT CCCTGACACC CCTACATTCA TATATTGAAT CACCCCTACT

GTGCTATGTC AGTATCTCCA GGTAAACCCT TCTCCCCTCC CCTATGTACG TCGTGCATTA

ATGGTTTGCC CCATGCATAT AAGCATGTAC ATAATATTAT ATCCTTACAT AGGACATATT

AACTCAATCT CATAATTCAC TGATCTTTCA ACAGTAATCG AATGCATATC ACTTAGTCCA

ATAAGGGCTT AATCACCATG CCTCGAGAAA CCATCAACCC TTGCTCGTAA TGTCCCTCTT

CTCGCTCCGG GCCCATACTA ACGTGGGGGT TACTATCATG AAACTATACC TGGCATCTGG

TTCTTACTTC AGGGCCATAA CTTTATTTAC TCCAATCCTA CTAATTCTCG CAAATGGGAC

ATCTCGATGG ACTAATGACT AATCAGCCCA TGATCACACA TAACTGTGGT GTCATGCATC

TGGTATCTTT TAATTTTTAG GGGGGGAATC TGCTATCACT CACCTACGAC CGCAACGGCA

CTAACTCTAA CTTATCTTCT GCTCTCAGGG AATATGCCCG TCGCGGCCCT AATGCAGTCA

AATAACTTGT AGCTGGACTT ATTCATTATC ATTTATCAAC TCACGCATAA AATCAAGGTG

CTATTCAGTC AATGGTTTCA GGACATATAG TTTTAGGGTA CACGTACGTA CACGTACGTA

CACGTACGTA CACGTACGTA CACGTGCGTA CACGTACGTA CACGTACGTA CACGTGCGTA

CACGTACGTA CACGTXCGTA CACGTGCGTA CACGTXCGTA CACGTGCGTA CACGTGCGTA

CACGTGCGTA CACGTCGTAA GACATTAAGT TAACTTATAC AAACCCCCCT TACCCCCCGT

AAACTCATGT CATCTATTAT ACACTTATTT ATGTCCCGCC AAACCCCAAA AACAGGACTA

AGTGCATACA ATACTCACAA GCTTTATTTA AATTATATAC AAATGTATTG CTACTCTAGT

TAACTTAACA CAACAGTCTT ACACGCATTT GGTCTCGTAG TCTATCTATA GATAGCATTC

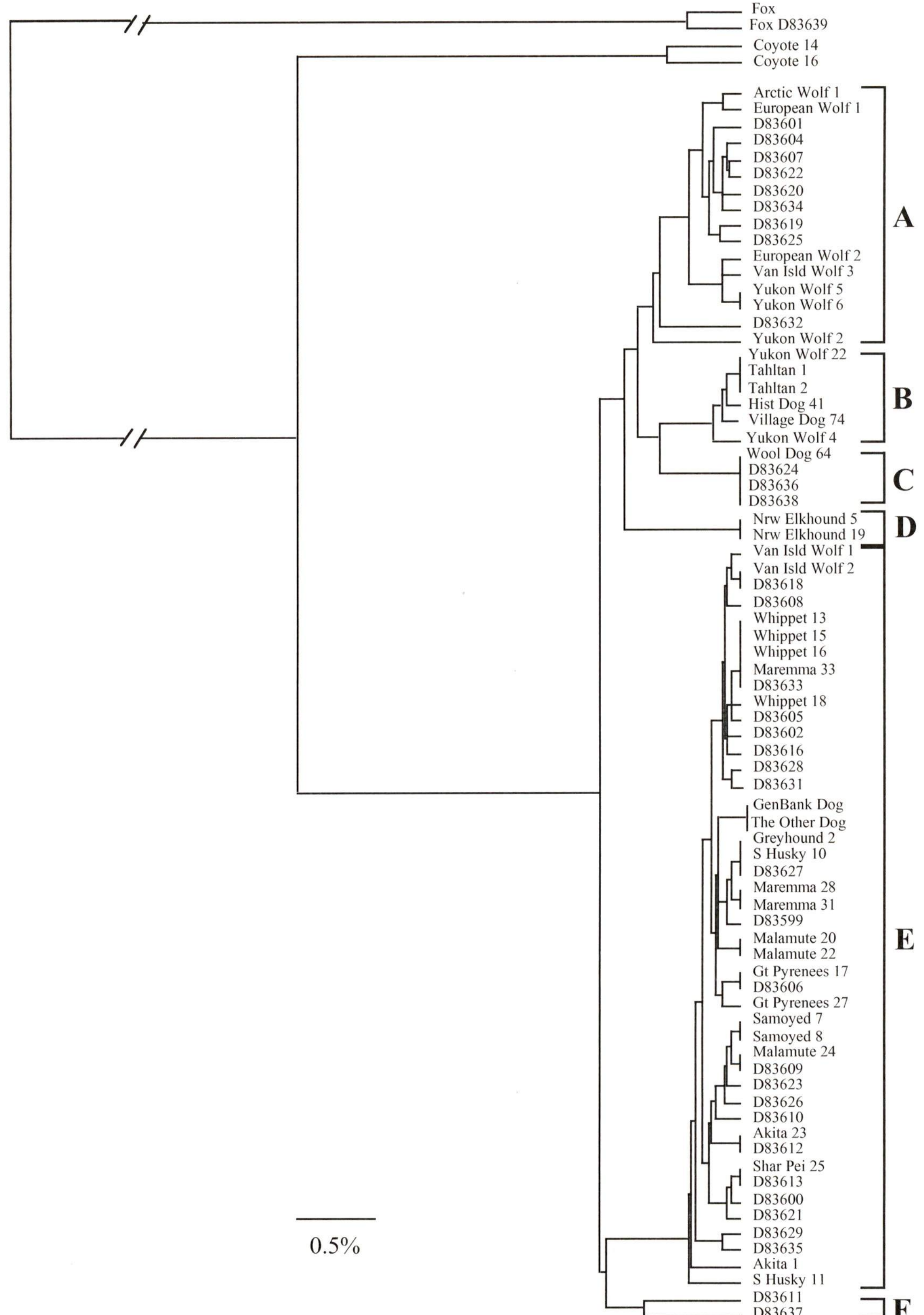
CCTTTTTTTT CCCTCTCATA TTTACTATGT ATTTTATTTA TTACGCACAC TACAATTTCA

GTATAAGTTA ATGTAGCTTA ATTAATAAAG CAAGGCACTG AAAATGCCAA GATGAGTCGC

ACTACTCCAT AXACATAAAG GTTTGGTCCC AGCCTTGGTA CC

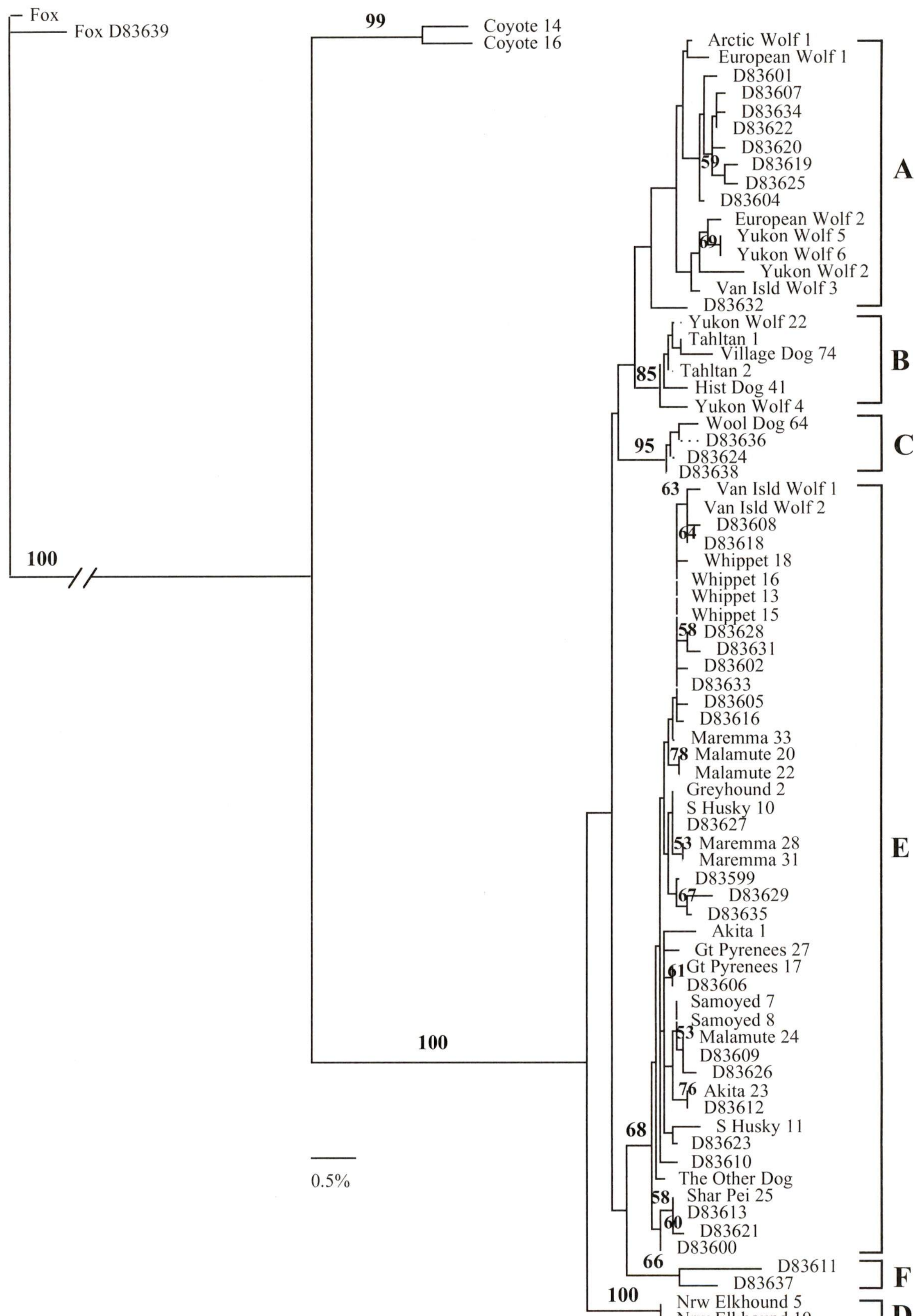
Fig. 3.2 Tandem repeats (underlined) in d-loop sequence of whippet 13. The repeats span a 150 bp segment of the d-loop and consist predominantly of the sequence GTACACGTAC, although the second to last base is found as either an A or a G.

Fig. 3.3 UPGMA trees of the sequence data generated by this study combined with Okumura *et al.*'s (1996) data. Okumura *et al*'s (1996) dog haplotypes are represented by accession number, the key to which is found in table 3.3. Other abbreviations found in the figure are Van Isld Wolf (=Vancouver Island wolf), Tahltan 1 (=Tahltan bear dog 2892), Tahltan 2 (=Tahltan bear dog 4758), Hist Dog (=historic dog), Nrw Elkhound (=Norwegian elkhound) and S Husky (=Siberian husky). Bolded letters beside the brackets designate the clusters described in the text; A="wolf", B=indigenous hunting dogs, C=wool dog and cohorts, D=Norwegian elkhound, E= modern dogs and F=some Japanese dogs.



Fox
Fox D83639
Coyote 14
Coyote 16
Arctic Wolf 1
European Wolf 1
D83601
D83604
D83607
D83622
D83620
D83634
D83619
D83625
European Wolf 2
Van Isld Wolf 3
Yukon Wolf 5
Yukon Wolf 6
D83632
Yukon Wolf 2
A
Yukon Wolf 22
Tahltan 1
Tahltan 2
Hist Dog 41
Village Dog 74
Yukon Wolf 4
B
Wool Dog 64
D83624
D83636
D83638
C
Nrw Elkhound 5
Nrw Elkhound 19
D
Van Isld Wolf 1
Van Isld Wolf 2
D83618
D83608
Whippet 13
Whippet 15
Whippet 16
Maremma 33
D83633
Whippet 18
D83605
D83602
D83616
D83628
D83631
GenBank Dog
The Other Dog
Greyhound 2
S Husky 10
D83627
Maremma 28
Maremma 31
D83599
Malamute 20
Malamute 22
Gt Pyrenees 17
D83606
Gt Pyrenees 27
Samoyed 7
Samoyed 8
Malamute 24
D83609
D83623
D83626
D83610
Akita 23
D83612
Shar Pei 25
D83613
D83600
D83621
D83629
D83635
Akita 1
S Husky 11
E
D83611
D83637
F
0.5%

Fig. 3.4 Neighbour Joining trees of the sequence data generated by this study combined with Okumura *et al.*'s (1996) data. Bootstrap values were computed excluding the short ancient sequences and are superimposed on the branches that are supported by values greater than 50. Okumura *et al*'s (1996) dog haplotypes are represented by accession number, the key to which is found in table 3.3. Other abbreviations found in the figure are Van Isld Wolf (=Vancouver Island wolf), Tahltan 1 (=Tahltan bear dog 2892), Tahltan 2 (=Tahltan bear dog 4758), Hist Dog (=historic dog), Nrw Elkhound (=Norwegian elkhound) and S Husky (=Siberian husky). Bolded letters beside the brackets designate the groups described in the text; A= "wolf", B=indigenous hunting dogs, C=wool dog and cohorts, D=Norwegian elkhound, E= modern dogs and F=some Japanese dogs.


Fox
Fox D83639
100
99
Coyote 14
Coyote 16
Arctic Wolf 1
European Wolf 1
D83601
D83607
D83634
D83622
D83620
59
D83619
D83625
D83604
European Wolf 2
69
Yukon Wolf 5
Yukon Wolf 6
Yukon Wolf 2
Van Isld Wolf 3
D83632
A
Yukon Wolf 22
Tahltan 1
Village Dog 74
85
Tahltan 2
Hist Dog 41
Yukon Wolf 4
B
Wool Dog 64
95
D83636
D83624
D83638
C
63
Van Isld Wolf 1
Van Isld Wolf 2
D83608
64
D83618
Whippet 18
Whippet 16
Whippet 13
Whippet 15
58
D83628
D83631
D83602
D83633
D83605
D83616
Maremma 33
78
Malamute 20
Malamute 22
Greyhound 2
S Husky 10
D83627
53
Maremma 28
Maremma 31
D83599
67
D83629
D83635
Akita 1
Gt Pyrenees 27
61
Gt Pyrenees 17
D83606
Samoyed 7
Samoyed 8
53
Malamute 24
D83609
D83626
76
Akita 23
D83612
S Husky 11
68
D83623
D83610
The Other Dog
58
Shar Pei 25
D83613
60
D83621
D83600
E
66
D83611
D83637
F
100
100
Nrw Elkhound 5
Nrw Elkhound 19
D
0.5%

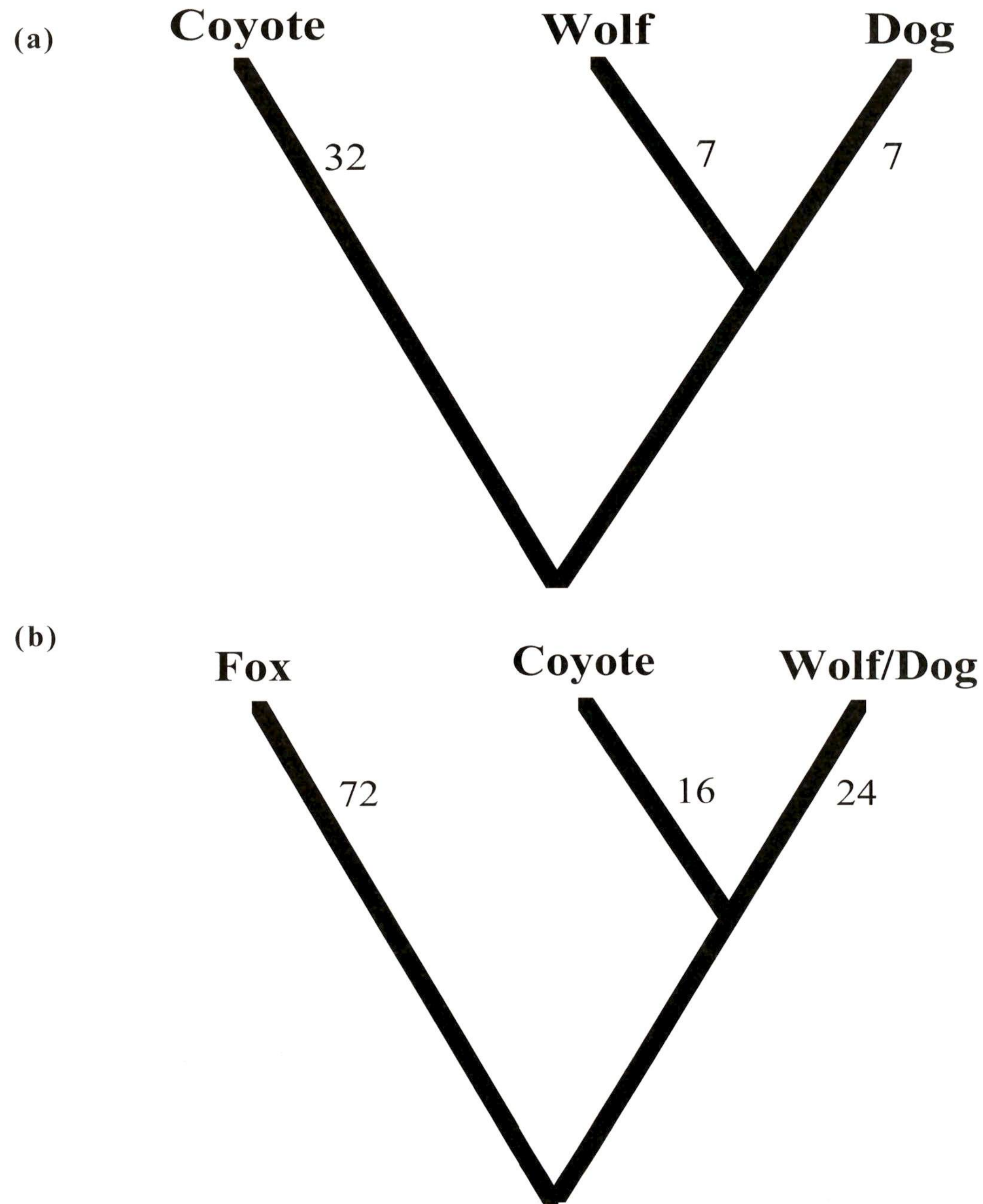
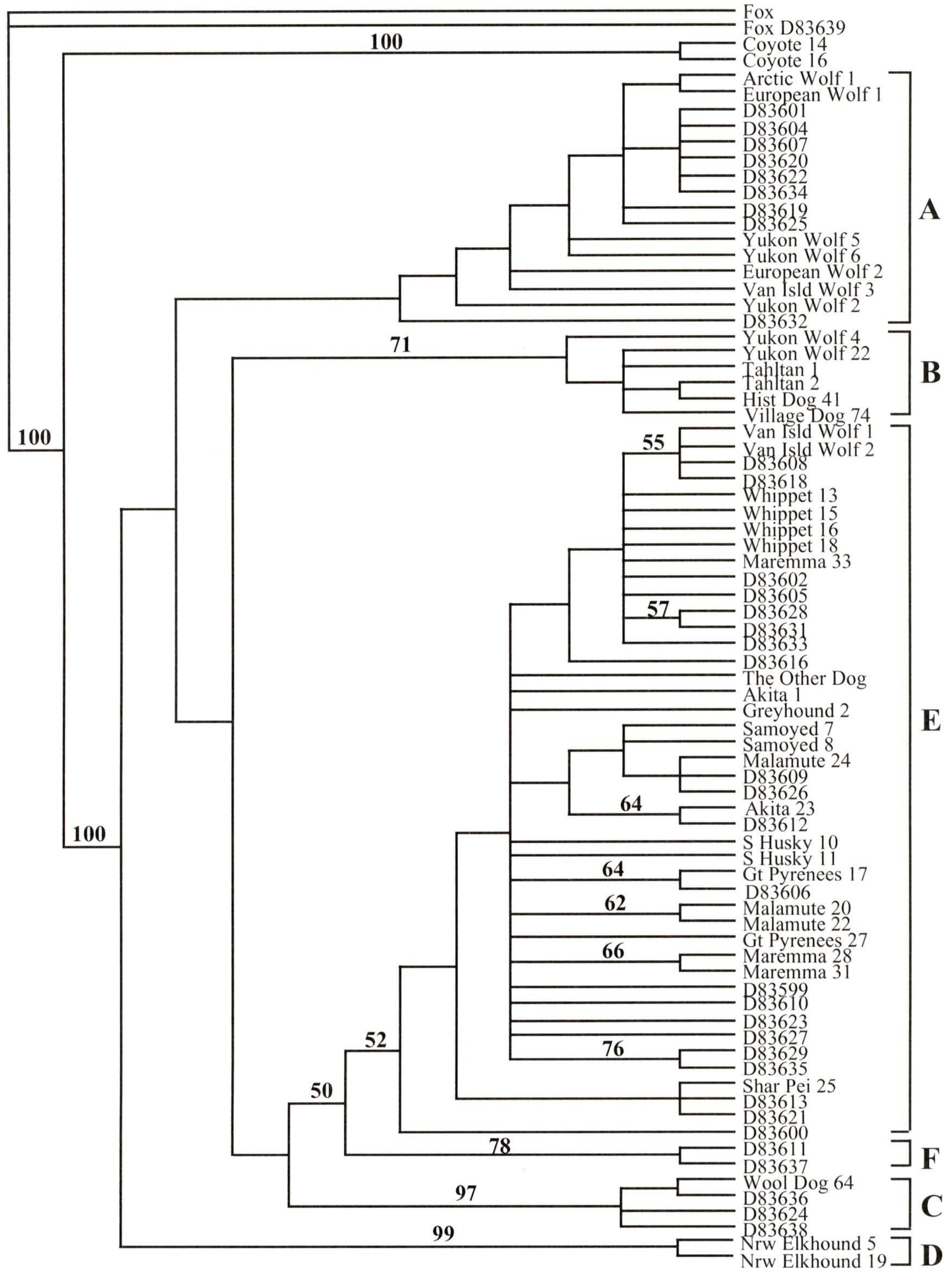


Fig. 3.5 Stylized trees showing results of the relative rate tests. Numbers of nucleotide substitutions since divergence are superimposed on the branches. **(a)** Dog versus wolf with coyote as the reference taxon and **(b)** dog/wolf versus coyote with fox as the reference taxon.

Fig. 3.6 Most parsimonious trees of the sequence data generated by this study combined with Okumura *et al.*'s (1996) data. Bootstrap values, excluding short ancient sequences, greater than 50 are included on the branches. Okumura *et al*'s (1996) dog haplotypes are represented by accession number, the key to which is found in table 3.3. Other abbreviations found in the figure are Van Isld Wolf (=Vancouver Island wolf), Tahltan 1 (=Tahltan bear dog 2892), Tahltan 2 (=Tahltan bear dog 4758), Hist Dog (=historic dog), Nrw Elkhound (=Norwegian elkhound) and S Husky (=Siberian husky). Bolded letters beside the brackets designate the clades described in the text; A= "wolf", B=indigenous hunting dogs, C=wool dog and cohorts, D=Norwegian elkhound, E= modern dogs and F=some Japanese dogs.


Fox
Fox D83639
Coyote 14
Coyote 16
Arctic Wolf 1
European Wolf 1
D83601
D83604
D83607
D83620
D83622
D83634
D83619
D83625
Yukon Wolf 5
Yukon Wolf 6
European Wolf 2
Van Isld Wolf 3
Yukon Wolf 2
D83632
Yukon Wolf 4
Yukon Wolf 22
Tahltan 1
Tahltan 2
Hist Dog 41
Village Dog 74
Van Isld Wolf 1
Van Isld Wolf 2
D83608
D83618
Whippet 13
Whippet 15
Whippet 16
Whippet 18
Maremma 33
D83602
D83605
D83628
D83631
D83633
D83616
The Other Dog
Akita 1
Greyhound 2
Samoyed 7
Samoyed 8
Malamute 24
D83609
D83626
Akita 23
D83612
S Husky 10
S Husky 11
Gt Pyrenees 17
D83606
Malamute 20
Malamute 22
Gt Pyrenees 27
Maremma 28
Maremma 31
D83599
D83610
D83623
D83627
D83629
D83635
Shar Pei 25
D83613
D83621
D83600
D83611
D83637
Wool Dog 64
D83636
D83624
D83638
Nrw Elkhound 5
Nrw Elkhound 19
A
B
E
F
C
D
100
100
100
71
55
57
64
64
62
66
76
52
50
78
97
99

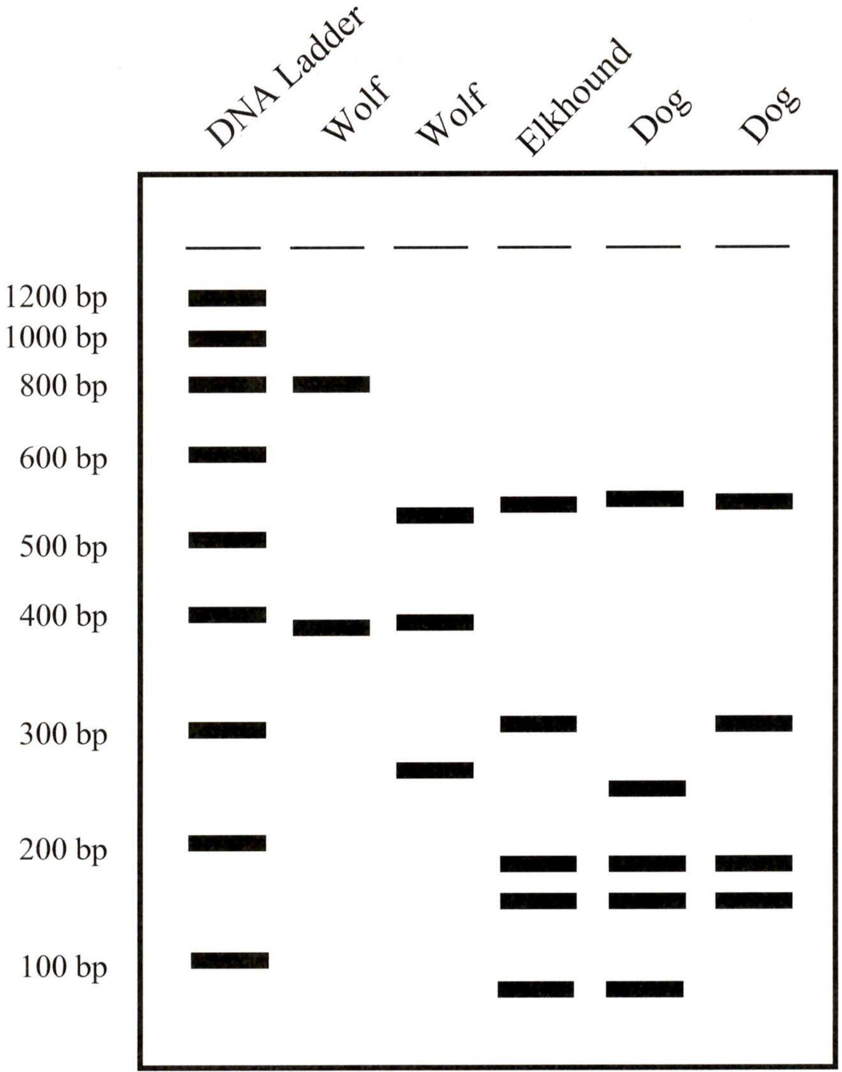


Fig. 3.7 Expected banding patterns from "quick test" described in text. Numbers on the side of the stylized gel indicate sizes of the DNA ladder fragments.

Discussion

4.1 Problems with Ancient DNA

The problems of PCR inhibition and contamination relating to DNA extracted from ancient tissues have been reported by several other authors (Pääbo *et al.* 1988; Hagelberg *et al.* 1989; Hagelberg & Clegg 1991). In this study it was sometimes impossible to amplify the d-loop target from ancient dog and wolf extracts that were confirmed to contain DNA. This is because ancient DNA is often damaged and modified by hydrolytic and oxidative processes which inhibit PCR (Lindahl 1993a; Höss *et al.* 1996). Another source of PCR inhibition is chemicals: fulvic and humic acids resulting from burial are often present in bone (Tuross 1994), and museum skins and mummified remains are often preserved with inhibitory chemicals (Pääbo *et al.* 1988). The other common problem with the ancient DNA was contamination. The repeated occurrence of contamination in the ancient DNA extraction controls and in PCR controls exemplifies the importance of utilizing such controls when employing ancient DNA techniques, as has been recommended by several authors (Pääbo 1989; Lindahl 1993a, 1993b; Handt *et al.* 1994; Hedges & Schweitzer 1995; Kringer *et al.* 1997; Yang, Golenberg & Shoshani 1997).

4.2 Complexities Involved in Wolf/Dog Studies

There is a lack of primary documentation to substantiate many of the current assumptions about dogs and their history, as is apparent from reviewing the literature for the present study. One assumption often made is that domestication of the wolf caused a speciation (or divergence) event. It is equally plausible though that wolf populations were diverging before domestication occurred because of behavioral differences. Some wolves may have been very timid of humans and perhaps others were drawn to human settlements (Morey 1994). Another popular belief is that dogs unique to a geographical region are descendants of the dogs found in local archaeological sites. However, to date, there are

few studies that morphologically compare archaeological dog remains to contemporary breeds. The only known examples are Gollan's study of the Australian dingo (1982) and Shigehara *et al.*'s (1997) comparison of the modern Japanese shiba inu skeleton to ancient Japanese dog remains. There are many assumptions about dogs and their history, but they are all difficult to substantiate without a larger data set.

There are several confounding factors which have to be considered when interpreting data relating to dog and wolf questions. One factor that has to be contemplated when using DNA data from modern breeds to explore dog history is that it has been shown (Okumura *et al.* 1996; Vilà *et al.* 1997; this study) that a substantial amount of crossbreeding has occurred in dogs. Crossbreeding has been both recorded and unrecorded, deliberate and undeliberate and has caused mitochondrial haplotypes to become nonspecific to breeds, hindering interpretation of phylogenetic data. Another problem is the definition of dog breeds throughout history, since the current concept of a breed is quite different than that of the past, as pointed out by Glyn (1967). Therefore, caution must be taken when interpreting early written and oral records. Still another confounding factor that studies (Vilà *et al.* 1997; this study) have found is that introgression occurs between dogs and wolves, and that the gene flow probably occurs in both directions. A related problem is that both dogs and wolves have been intentionally and accidentally moved around the globe by humans. This would cause the phylogeographic structure of ancient introgression to be unnaturally dispersed throughout continents. This problem is not normally encountered in other studies which use mitochondrial DNA to trace migration patterns of animal groups, but has occurred here because of man's unique relationship with the dog (Clark 1995). A final complication to consider is that there are an unknown number of domestication events from an unknown founder population (or populations) of wolves, or from a wolf ancestor. All of these factors contribute to the challenge of deciphering the true history of dogs.

By selecting stock breeds, from which all other breeds were likely developed, it was hoped that the history of major dog lineages could be discovered through their mitochondrial DNA. Even though the dog breeds in this study are morphologically and behaviourially distinct and are thought to have relatively pure histories (over several thousand years), all of these modern breeds and the majority of the Japanese dogs are genetically indistinct with respect to the d-loop. (As was previously mentioned, the term "modern dogs" within this discussion refers to the dogs in group E of the phylogenetic trees. This group includes all modern dog breeds with the exception of the Norwegian elkhounds and some of the Japanese dogs.) Morphological distinctness among the breeds is probably a result of heterochrony (Wayne 1986a). If we are to believe that these breeds were at one time genetically unique, they must have been subjected to extensive interbreeding in their recent history. Some breeds, like the akita and shar-pei, are known to be problematic because they experienced population bottlenecks prior to being reestablished from a few individuals. A similar situation has occurred with some other breeds that were developed (or redeveloped) from a breeding stock of individuals that had been exported from their native lands to Europe (e.g. samoyed, greyhound). The descendants of these individuals are the ones that were exported to the rest of the world, registered with the kennel clubs, and appear to have been the ones included in this study. Most breeds are problematic for phylogenetic analysis because they have been recently crossed with other breeds to introduce new characteristics for "improvement," or they have been created by crossing two or more older stock breeds. With all of that gene manipulation, it is perhaps not surprising that one cannot distinguish genetically among dog breeds.

Vilà *et al.*'s (1997) recent study examines several breeds that are thought to have older origins, as well as many "new" breeds and crossbreeds. The old breeds that they included were: the afghan hound, a supposedly ancient greyhound breed; the basenji,

purported to be the oldest pure African breed; the Australian dingo, alleged to be an ancient purebred descended from the Thai dog; the xolo (Mexican hairless), thought to have migrated from Asia to South America with the Aztecs; more individuals of Norwegian elkhound from both North America and Scandinavia; two closely related Scandinavian breeds, the jämthund (or Swedish elkhound) and the Norwegian buhund, and the Norwegian lundehund. They also included more wolves, with worldwide geographic distribution. Two preliminary parsimony analyses of the Vilà *et al.* (1997) data combined with those of the present study and Okumura *et al.*'s (1996) produced cladograms (fig. 4.1 and data not shown) which support the relationships of the major clades in fig.s 3.3, 3.4 and 3.6. The majority of the Vilà *et al.* dogs fell within the modern dog clade (E), including some of the Scandinavian breeds, crossbreeds and all of the older breeds. With a few exceptions, the addition of these samples to the analyses did not help elucidate many of the questions pertaining to domestic dog origins.

4.3 Domestication Events Supported by the Current Data

One hypothesis that can be drawn from all of the dog data is that multiple domestication events may have occurred in the history of the dog, one involving most modern breeds (E), a second involving the wool dog and some Japanese dogs (C), a third involving the North American indigenous hunting-type dogs (B) and a fourth event involving the Norwegian elkhound (D). Previous allozyme data (Juneja & Shibata 1992) suggest that at least two domestication events occurred involving European (e.g. the modern dog clade E) and Asian (e.g. the wool dog and cohorts clade C) dogs.

The location of a domestication event which would have led to the modern dog clade is unknown. As a group, the modern dogs retain haplotypes that are marginally distinct from those of the wolves. This implies that the purebreeding of dogs for the show dog circuit has reproductively isolated these animals from wolves to some extent. This

seems logical when one considers that the majority of humans who breed and own purebreds control the breeding of their animals. On the other hand, one may postulate that intensive interbreeding among kennel registered breeds has caused the problems in finding genetic breed markers.

The second and third possible domestication events involved the extinct North American indigenous dogs. These dogs may have retained the mitochondrial haplotype of prehistoric wolf populations from which they were domesticated, although determining whether the dogs were domesticated in Asia or North America is not possible with the current data set. Given the close relationship of the wool dog to some of the Japanese dogs, a domestication event seems to have occurred in Asia before the ancestor of the wool dog was brought to North America. The two groups of North American indigenous dogs may have been domesticated from the same wolf stock in Asia, but have followed different evolutionary paths due to their reported different uses. Domestication of dogs on the Northwest coast does not seem likely though, since all other evidence to date indicates that dogs were brought to North America by early human immigrants as fully domesticated animals (Lawrence, 1967, 1968; Olsen & Olsen, 1977; Olsen, 1985; Morey & Wiant, 1992).

A fourth potential domestication event involved the Norwegian elkhound. Examination of the hypervariable region of the d-loop reveals that the ancient indigenous dogs and Norwegian elkhounds shared no unique sites, suggesting that the two groups arose from different ancestral populations of wolves. Based on the number of retained fox, coyote and wolf characters, as well as unique characters, the elkhound diverged very early from the modern dogs. The separate domestication events of the modern dogs and elkhounds also seems to be supported by Vilà *et al.*'s (1997) data, as two of their elkhound haplotypes (D8a and D8b) are very similar to those of the present study and all are closely related to Vilà *et al.*'s (1997) wolves from France, Italy (W4), Greece and Romania (W5).

This result supports the distinctness of at least one elkhound lineage from the rest of the dogs. There are several possible interpretations of the phylogenetic relationship between the modern dogs (E) and the elkhounds (D): 1) both groups of dogs may have diverged very early from a primitive dog that predated the appearance of modern wolves (*Canis lupus*); 2) the ancestral elkhound and other dogs may have diverged from the same wolf stock, but the elkhound lineage split off much earlier, or 3) the ancestral elkhounds and other dogs were domesticated from separate wolf subspecies. The last scenario would fit Clutton-Brock's (1984) hypothesis of multiple domestication events, but does not relate to her morphological groupings. This preliminary study of mitochondrial DNA points towards the possibility that several other canine domestication events occurred throughout the world. The alternative hypothesis to multiple domestication events is that all the dogs, except the modern dogs (E), have been back-breeding to wolves, and therefore are not pure dogs (see section 4.4).

4.4 Wolf Relationships and Introgression with Dogs

As was seen in the phylogenetic trees (figs. 3.3, 3.4, 3.6 and 4.1), the wolves do not partition according to subspecies. This can be attributed to the wolf's high mobility, wide distribution and to the translocation of wolves by humans in the interests of conservation. Wolves can move great distances (Mech 1995) and are the most widely distributed large mammal after humans (Clutton-Brock 1984). However, they tend not to disperse if the need does not arise (Friis 1985). Dispersal of North American wolves did occur during the Pleistocene glaciation; some populations moved into Asia (Olsen 1985), while others moved to the south (Hall 1978). Since it is unlikely that multiple, nearly identical haplotypes have arisen repeatedly in geographically separated areas, translocation of wolves is the most parsimonious explanation of the close relationship of the Arctic and European wolf haplotypes. Several reintroduction programmes have caused the transport

of individuals (wolves and others) from healthy populations to sparsely populated areas in an effort to reestablish those dwindling or decimated populations (Conant 1988; Griffith *et al.* 1989; Mech 1995; Fritts *et al.* 1997). The mitochondrial haplotypes then become more widely dispersed than they would under natural conditions, sometimes being distributed across multiple continents. One should take this into account when using mitochondrial DNA to study biogeography, so that the results are comprehensible and lead to valid conclusions.

Another interesting finding was the evidence of introgression between dogs and wolves, which could also lead to 'strange' dispersal patterns of the mitochondrial genome. Introgression occurs sporadically in many animal taxa but among vertebrates it seems to be most prominent in fish, where it accounts for some seemingly odd dispersal patterns (Avise 1994). The introgression between wolves and dogs is most apparent within the northern Vancouver Island wolf samples. Friis (1985) reported morphological evidence of hybridization between dogs and wolves on southern Vancouver Island. The introgression is recent because wolves retain mitochondrial haplotypes that are identical and nearly identical to some of the dogs in this study (i.e. insufficient time has passed for mutations to accumulate in the d-loop). Gottelli *et al.* (1994) arrived at similar conclusions when examining the occurrence of hybridization of Ethiopian wolves (*Canis simensis*) and dogs. Evidence of introgression with wolves also was found with the Japanese dogs and Vilà *et al.*'s dogs, thus demonstrating that exchange of the dog and wolf genomes occurs in Europe and North America as well as Asia. Some of these dogs fell within the wolf clade, implying that gene flow occurs in the other direction as well (wolf to dog). Alternatively, this is a result of a very old introgression event where the dog mitochondrial genome flowed back to the wolves and has become pervasive among modern wolves. Perhaps the genes have been flowing in both directions throughout the history of the dog.

The ancient DNA data combined with the rest of the current dog and wolf data seem

to support either two domestication events within the North American indigenous dogs (see previous section) or one domestication event and the occurrence of introgression between the ancient North American hunting-type dogs and wolves. According to Mengel (1971), "...if hybrids are kept under semidomestic conditions, as by [North American indigenous peoples] there is abundant evidence of wolf genes introgressing into dog stocks." Walker and Frison (1982) describe a group of dogs indigenous to the American Plains as intermediary between the large eskimo type dog and wolves. The dogs that they examined were of the Plains-Indian type described by Allen (1920), the type to which the Coast Salish village dog corresponds (Crockford 1997a). According to Walker and Frison's (1982) morphological discriminant analyses of archaeological specimens, introgression occurred among several Plains indigenous dog populations and wolf populations over a minimum of six thousand years. It is also possible that introgression occurred in Asia before both ancient wolves and ancient dogs crossed the Bering Strait land bridge.

Vilà *et al*. (1997) found genetic evidence that a similar situation may have occurred in Europe with the Scandinavian elkhounds. Some of the Norwegian and Swedish elkhounds (D8a and D8b) retain a unique haplotype which is closely related to those of some wolves from France, Italy, Greece and Romania (W4 and W5). This could suggest that these dogs have hybridized with wolves close to their geographical region. However, it cannot be determined which direction the genes flowed. It seems likely that the elkhounds are a result of a unique domestication event and later introgressed with local wolves in the same manner as occurred on Vancouver Island.

Many complex issues arise in conservation biology relating to wolves in light of all the new dog and wolf data. If many modern wolves are result of introgression with dogs, what implications does that have for conservation efforts? Roy *et al.* (1996) suggested that conservation efforts should not be a high priority for the very endangered red wolf because these animals are a hybrid of grey wolf and coyote. How does one measure the value of a

hybrid? Are they worth conserving? Should we conserve now and ask questions later, or ask questions now and risk the loss? One has to consider the economic side of the debate also; ideally the first scenario would be favourable to most conservationists, but economically, the second situation predominates. These are philosophical conservation issues which are difficult to resolve objectively.

4.5 Phylogenetic Affinities of the Extinct Coast Salish and Tahltan Dogs

The extinct indigenous dogs seem to retain two different kinds of haplotypes. The hunting dogs, Tahltan bear dog, Coast Salish village dog and Coast Salish historic dog share nearly identical haplotypes with each other and with Yukon wolves 22 and 4. When all data are analysed, these dogs fall into a wolf clade which includes wolves from the present study as well several from Vilà *et al.*'s (1997) study (data not shown). These include wolves from Spain (W1 and W3), Portugal (W1 and W2), Croatia (W2), Greece (W2), Sweden (W2 and W10), Turkey (W2), Poland (W3), Bulgaria (W7), Israel (W7, W11 and W14), Estonia (W10), Russia (W10), Finland (W10), China (W14 and W27), Afghanistan (W18), Alberta (W21) and the Yukon (W21, W23 and W24). This result exemplifies the distinctness of the indigenous hunting dogs from all extant dogs. The Coast Salish wool dog haplotype differs substantially from the other indigenous dogs and is more closely related to three of the Japanese dogs and two of Vilà *et al.*'s dog haplotypes, D7 (German shepherd, Alaskan husky, Siberian husky, Swedish elkhounds, schnauzer, Airedale terrier and west highland terrier) and D21 (Mexican hairless). These individuals are all likely descended, through intercrossing of breeds, from the same ancient lineage as the wool dog. These data suggest that either two different domestication events occurred within the North American indigenous dogs, or that introgression occurred within one of the two groups of indigenous dogs (see previous sections for discussion of both of these scenarios). The wool dog would have retained its mitochondrial information

because, according to many early explorers and ethnologists, these dogs were kept separate from the hunting dogs (Schulting 1994 and references therein; Crockford 1997a). Whether the two clades of indigenous dogs originated from two separate domestication events or via introgression within one lineage, historical records that document that the Coast Salish kept their two dog types separate seem to be supported by data presented here, as well as those of Crockford (1997a).

4.6 Species Concepts and Taxonomic Classification of Dogs and Wolves

Based on the genetic data to date (Vrana *et al*. unpublished data; Wayne & O'Brien 1987; Wayne 1993; Vilà *et al.* 1997; this study), the validity of the classification of dogs and wolves into different species categories is questioned. However, all of the present genetic data are derived from the mitochondrial genome and there could be other differences in data obtained from the nuclear genome. The nuclear sites that most likely would be affected would be the genes involved in reproductive hormone production (Langridge 1991).

The classification of dogs and wolves as separate species must be examined in the light of major current and past species concepts. Past concepts are important in understanding why dogs and wolves were originally classified as separate species by Linnaeus in his 1758 *Systema Naturae* (Clutton-Brock 1984). Two species concepts will be discussed, the typological and biological concepts. Several others, such as the evolutionary species concept to delimit chronospecies, do not apply to the dog-wolf question. The typological species concept dates back to the ancient Greeks. The underlying idea of the concept is that each organism (and inanimate object) contained an essence (or *eidos*) that was shared with other organisms of its type (or species). Within a species, variations from the "type" were considered as flaws in the individual. In practice, organisms were classified into species categories according to morphological differences

from other species. Morphologically, wolves and most dogs are normally considered as quite distinct, so according to this species concept the two should be classified as separate species. However, Wayne (1986a) found that dogs and wolves were not distinct when he applied allometric analyses to the cranial morphology of dogs and several wild canids. He did find tremendous variation within the dogs and points out that differences in proportion and size between some breeds is as great as that between different genera in the wild (Wayne 1986a). A Boston terrier with its short snout and stature hardly seems morphologically similar to a long-snouted, tall and lean greyhound. Locke expressed this in his inability to see why two breeds of dogs "are not as distinct species as a spaniel and an elephant..." (Mayr 1982).

Theoretically, few biologists today favour the typological species concept (Mayr 1982), although it continues to be used for taxonomic purposes. The major problem with the typological species concept is that genetic diversity and morphological diversity are often uncorrelated, so that differences in evolutionary history are not always recorded by morphology (Wake *et al*. 1983; Cockburn 1991). This is exemplified by numerous recent studies which have shown the existence of genetically cryptic species among morphologically similar or identical sympatric populations (see reviews in Avise 1994, and Mayr 1996). The dogs and wolves seem to be on the opposite side of this cryptic species problem: the two species are usually considered to be morphologically distinct (Clutton-Brock 1984), but they are not genetically distinct. Morphological evolution of dogs is more akin to animals such as cichlid fishes where the rate of change in morphology can be up to 1,000 times as great as that in molecules of comparable adaptive significance (Langridge 1991). This would cause one to question the validity of the typological species concept, since one would be forced to classify two ecotypes of one species as two separate species (Mayr 1996). Historically, dogs and wolves were defined as distinct species according to the typological species concept; however, this classification should be

reexamined using the more recent biological species concept.

Dobzhansky's biological species concept relies on reproduction rather than on morphology: "Species are groups of actually or potentially interbreeding populations, which are reproductively isolated from other such groups." (Mayr 1942). Reproductive isolation and hybrid sterility are not the only criteria in delimiting species; some species may hybridize in captivity producing fertile offspring, but they are not found to do so in nature (Futuyma 1986). (It is interesting to point out that reproductive isolation appears to exist within the dogs. It seems impossible or at least difficult for some breeds to mate naturally, such as crossing a chihuahua to a Tibetan mastiff or to a harlequin great Dane.) Dog experts maintain that dogs and wolves were good biological species, despite interfertility, because of the morphological distinctness, and because hybridization of the two in nature was rare (Mengel 1971; Clutton-Brock 1984). This argument had its basis in Short's (1969) assertion that hybridization among good biological species is fairly common, though at moderate frequencies. However, evidence from the present study and that of Vilà *et al.* (1997) demonstrates that dogs and wolves do interbreed rather extensively in nature (see section 4.4), suggesting that the two are not separate species according to the biological species concept. Species limits are arbitrary in many cases, especially when dealing with differences that have occurred via anagenesis: where does one delineate the change from one species to another? This question is easier to deal with in terms of cladogenesis, since distinctive changes often define the origin of new species. One could venture that the definition of dog and wolf as separate species is more akin to the designation of palaeontological species that are the result of anagenesis. Species such as these (e.g. *Homo erectus* and *H. sapiens*) are named on bases of morphological distinctness rather than on reproductive isolation (Futuyma 1986).

"Conceptually, individuals are members of the same species if their genes could descend through the generations to unite in the same individual under natural conditions"

(Futuyma 1986). In the case of wolves and dogs though, it is unknown whether hybridization was natural or human-induced. This is particularly apparent with the Japanese data: mitochondrial haplotypes that are present in North American wolves are very similar to those of some Japanese dogs. This implies that the genes from a dog in Japan have ultimately united with those of a wolf in the Yukon, as Futuyma (1986) suggests could happen in white-tailed deer (*Ododoileus virginianus*) of Colorado and Florida. One might suggest that wolflike breeds (akita, malamute, Siberian husky, etc.) represent the hybrid zone between dog and wolf. These breeds are morphologically more similar to wolves than to other dogs; therefore, one might expect the mitochondrial haplotypes of these breeds to fall within the wolf clade more often than dogs of other breed groups. Haplotypes of other breeds also would be expected to fall into the wolf clade through unmonitored crosses with the spitz breeds. The only way that a new mitochondrial haplotype can enter a population (or clade, etc.) is via mutation or immigration. Since it is unlikely that multiple nearly identical haplotypes have arisen in several canine populations and "species," then immigration must explain these occurrences.

In the case of purebred "show" dogs, the immigration of new genetic material is predominantly influenced by humans, but also can be a result of the natural mating process (accidental breeding which results in "mutts" or crossbreeds or less desirable purebred pups). This artificial selection within dogs (and other domestic species) can increase morphological diversity while maintaining or reducing genetic diversity (Wayne 1986a). This explains why there is morphological diversity within the modern dog clade even though there is a lack of genetic variation. If this group of similar haplotypes originated in Europe, from where most of these breed lines were "discovered," it would be expected that the similar haplotypes would predominate in dogs samples from there as well. In fact, most of Vilà *et al.*'s (1997) dogs sampled from both Europe and North America fall into this modern dog clade (data not shown).

Artificial selection, which is responsible for the hundreds of dog breeds, is at the basis of Darwin's theory of natural selection (Futuyma 1986; Langridge 1991). The major differences between the two processes are that natural selection maintains or reduces phenotypic variation (Lewontin 1974), while artificial selection increases phenotypic variation and decreases genetic variation (Wayne 1986a; Langridge 1991). However, valuable information can still be acquired from studies of artificial selection which can be used to understand evolution (Futuyma 1986). In dogs, characteristics that are artificially selected are paedomorphic characteristics of wolves, such as tameness, playfulness, short snout and other puppy-like cranial characteristics, or cuteness (Morey 1994; Clutton-Brock 1995). The selection of these characteristics has resulted in morphological and behavioral changes (Clutton-Brock 1995). The reproductive rate also has increased in dogs because of hormonal changes induced by heterochrony (Wayne 1986a; Clutton-Brock 1995). Studying the hormonal changes that govern these changes allows one to gain insight into the relationships between the endocrine system and development and how the two can influence the evolution of species (Langridge 1991). One also can gain insight into evolutionary processes through comparative studies of phenotypic plasticity (the range of phenotypes that a genotype can produce) in dogs and wild animals (Wayne 1986a). Phenotypic diversity in natural populations arises in response to environmental pressures acting on available genetic material (Vrba & Eldredge 1984; Brandon 1996). One can examine the limits of this plasticity via comparisons to plasticity in dogs and other domestic animals which have been subjected to controlled selection. Perhaps different dog breeds could be considered as different ecotypes of *Canis familiaris*, if one could correlate morphological characteristics with the supposed geographical and cultural origins of the breeds. Artificial selection also can be used to understand convergent evolution, the process by which animals that are adapted to similar niches in geographically separated areas become morphologically similar even though unrelated. This can be demonstrated by

the similarities of the elkhounds to the other northern spitz breeds. All of these spitz breeds are intelligent, strong, have keen hearing and double coats, all characteristics needed for a freighting and hunting dog in the frigid northern climates. These traits were selected in different geographic areas of origin and resulted in convergent evolution. A similar process in nature results in morphologically identical cryptic sister species (Mayr 1996) and in less closely related but morphologically similar species such as the wolf and the marsupial wolf (Thomas *et al.* 1989). These are a few examples of how the study of artificial selection can be useful in questions relating to natural selection and evolution.

Ancient DNA technology can also be used to answer perplexing questions relating to evolutionary biology. A great benefit of ancient DNA is that it can be used to resolve some of the species concept problems regarding extinct species that were morphologically similar but were actually reproductively isolated (Cooper 1994; DeSalle 1994). Theoretically, one can determine if populations of relatively recently extinct organisms were interbreeding or reproductively isolated, and thus apply the biological species concept to these groups. These ancient DNA techniques can be applied to studies involving extant species as well, in order to check or calibrate the divergence time of groups estimated by the molecular clock. In this study, ancient DNA confirms the close association of dogs and wolves as far back as 4,000 years BP and helps demonstrate that the history of the dog likely encompasses multiple domestication events as well as multiple introgression events. Ancient DNA techniques now allow biologists to test evolutionary questions that could only be discussed theoretically in the past.

4.7 Summary

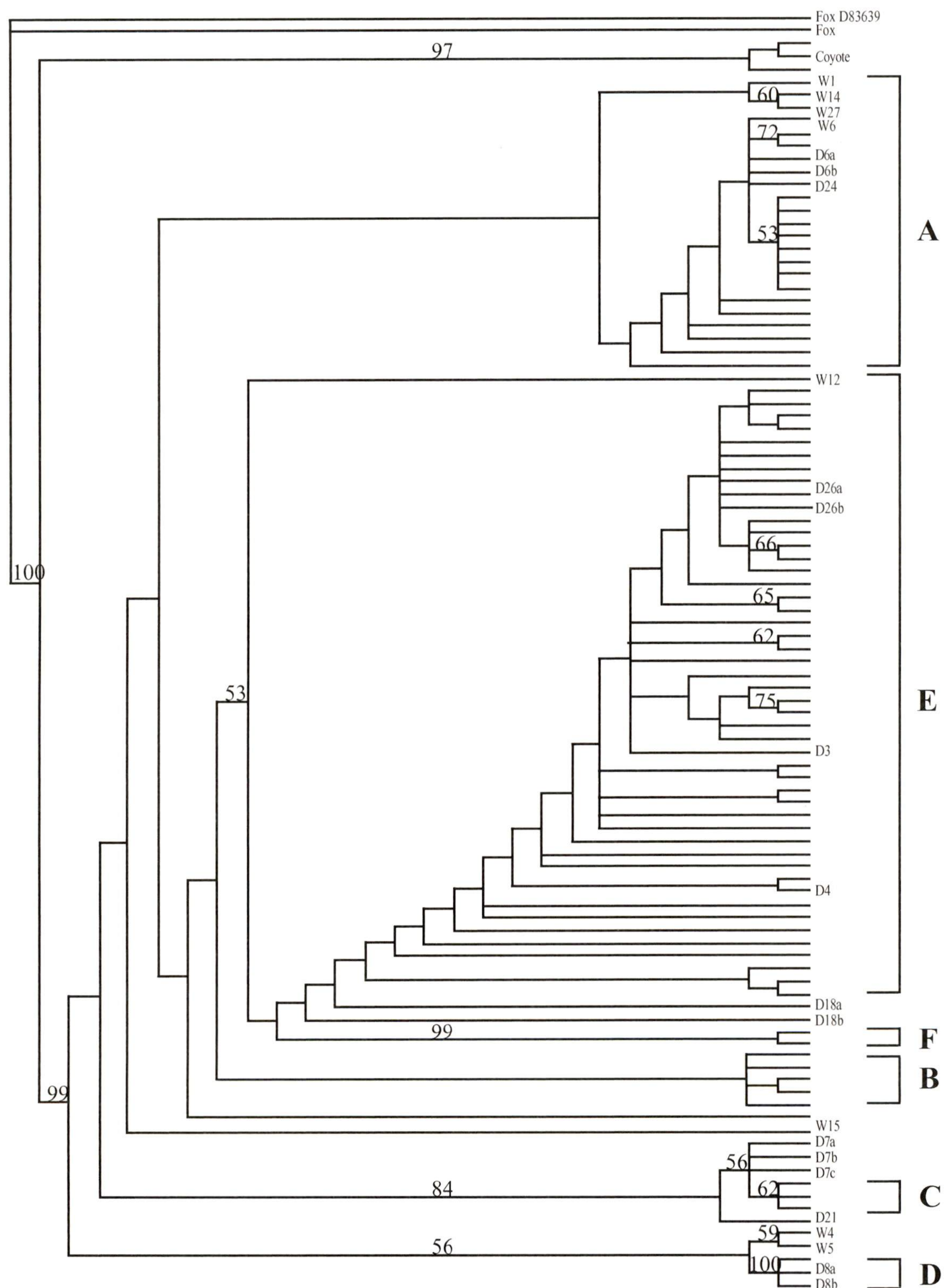
The mitochondrial data from the present study, combined with that of Okumura *et al.* (1996) and Vilà *et al.* (1997), revealed no markers that would allow distinction among dog breeds. Nor were markers found that could distinguish among the four major groups

of dogs. The data clearly demonstrated, however, that dogs and wolves are very closely related, and that fox and coyote are genetically distinct from each other and from the dog/wolf group. Perhaps the use of microsatellites could provide pedigree specific markers for dog breeds, but the present evidence suggests extensive gene flow among breeds. Nuclear genes which are affected by domestication, such as hormonal genes, may be worthwhile targets for future studies of dog and wolf relationships.

The data seems to support the occurrence of multiple domestication events during the history of the dog; however, the current data set cannot be used to determine the geographic origin of these events. It is also unknown as to whether dogs were domesticated from *Canis lupus* or from an extinct common ancestor of wolves and dogs. Whatever the case, this study and that of Vilà *et al.* (1997) present very strong evidence that wolves and dogs naturally interbreed, in that dogs were found in "wolf" groups and wolves were found in "dog" groups within the phylogenetic trees. Therefore, according to the biological species concept, *Canis familiaris* and *C. lupus* are not good species, even though they are generally accepted as such according to the typological species concept. Future studies could address the extent of wolf-dog introgression in specific cases, as well as the extent of hybridization between dogs and other canids.

The evidence presented in this study, as well as that of Crockford (1997a), seems to support the ethnographic reports that the Coast Salish peoples kept two distinct dog types, the wool dog and the village dog. However, all of the Northwest Coast dogs sampled are closely related to wolves. The North American dogs were probably domesticated in Asia and later interbred with wolves on this continent. To further investigate the geographic origins of these dogs, more data would need to be collected from ancient eastern Asian dogs as well as ancient wolf populations of both eastern Asia and western North America.

Fig. 4.1 Most parsimonious tree of all dog and wolf d-loop sequence data which exceed 717 bp (Okumura *et al.* 1996, Vilà *et al.* 1997 and this study).Taxon names from Vilà *et al.* (1997) only are labelled; all other taxa are the same as those found in fig. 3.3. Again, bolded letters beside the brackets designate the clades described in the text; A= "wolf", B=indigenous hunting dogs, C=wool dog and cohorts, D=Norwegian elkhound, E= modern dogs and F=some Japanese dogs. Bootstrap values greater than 50 are included on the branches. The wolf mitochondrial haplotypes in Vilà et al. (1997) used in this analysis were W1 (Portugal and Spain), W4 (France and Italy), W5 (Greece and Romania), W6 (Romania and Russia), W12 (India and Israel), W14 (China and Israel), W15 (Israel), and W27 (China). Their dog haplotypes that were included were D3 (chow chow, Norwegian elkhound, Mexican hairless, Siberian husky, papillon, poodle, rottweiler, English setter, Icelandic sheepdog, springer spaniel, Japanese spitz, border terrier, fox terrier, whippet, crossbreeds), D4 (boxer, German shepherd, Alaskan husky, kuvasz, Leonberger, Newfoundland, papillon, flat-coated retriever, golden retriever, Labrador retriever, samoyed, schipperke, giant schnauzer, Norfolk terrier, crossbreeds), D6 (German shepherd, groenendael, Mexican hairless, afghan, maremma, otter hound, toy poodle, golden retriever, Irish water spaniel, Tibetan spaniel), D7 (German shepherd, Alaskan husky, Siberian husky, Swedish elkhounds, giant schnauzer, Airedale terrier and west highland terrier), D8 (Norwegian elkhound, Swedish elkhound), D18 (dingo, Siberian husky, New Guinea singing dog, crossbreeds), D21 (Mexican hairless) D24 (golden retriever) and D26 (Mexican hairless, pug, Rhodesian ridgeback, shar). The second analysis of all available canid d-loop sequences was based on a much smaller 250 bp region of all available canid data (data not shown).


Fox D83639
Fox
Coyote
W1
W14
W27
W6
D6a
D6b
D24
W12
D26a
D26b
D3
D4
D18a
D18b
W15
D7a
D7b
D7c
D21
W4
W5
D8a
D8b
A
E
F
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Appendix A

Following is the key for the PCR method column in table A-1. The number in the column corresponds to one of the six PCR reaction mixtures listed, and the letter corresponds to one of the PCR machine programmes.

PCR reaction mixtures:

<u>1</u>
1X PCR buffer
200 µM each dNTP
0.5 µM each primer
1.0 unit *Taq*

<u>2</u>
1X PCR buffer
200 µM each dNTP
0.5 µM each primer
1.0 units *Taq*
50 µg/mL BSA

<u>3</u>
1X PCR buffer
200 µM each dNTP
0.5 µM each primer
1.0 units *Taq*
20 µg/mL BSA

<u>4</u>
1X PCR buffer
200 µM each dNTP
0.5 µM each primer
1.0 units Pharmacia *Taq*
20 µg/mL BSA

<u>5</u>
1X PCR buffer
200 µM each dNTP
1.0 µM each primer
2.0 units *Taq*
40 µg/mL BSA

<u>6</u>
1X PCR buffer
200 µM each dNTP
1.0 µM each primer
2.0 units Pharmacia *Taq*
40 µg/mL BSA

<u>7</u>
1X PCR buffer
200 µM each dNTP
0.5 µM each primer
2.0 units Pharmacia *Taq*
20 µg/mL BSA

<u>8</u> (Thomas *et al.* 1990)
67 mM Tris-HCl, pH 8.8
2.0 mM $MgCl_2$
10 mM B-mercaptoethanol
250 µM each dNTP
0.25 µM each primer
2.0 µg/mL BSA
2.0 units Pharmacia *Taq*

<u>9</u> (Thomas *et al.* 1990)
67 mM Tris-HCl, pH 8.8
2.0 mM $MgCl_2$
10 mM B-mercaptoethanol
250 µM each dNTP
0.25 µM each primer
2.0 µg/mL BSA
2.0 units Gibco *Taq*

Programmes:

<u>A</u>
94°C 5'
94°C 45" \
50°C 45" | 40X
72°C 90" /
72°C 7'

<u>B</u>
94°C 4'
94°C 45" \
50°C 45" | 35X
72°C 2' /
72°C 7'

<u>C</u>
94°C 5'
94°C 45" \
50°C 45" | 40X
72°C 2' /
72°C 7'

<u>D</u> (Thomas, *et al*)
94°C 4'
92°C 3' \
50°C 1' | 40X
72°C 1' /
72°C 7'

Table A-1 Success of the different PCR methods used to amplify ancient DNA.

Method	Success	Method	Success	Method	Success
1A	none	3C	none	7B	low
1B	low	4B	none	8D	low
2B	none	5B	none	9D	good
3B	moderate	6B	low	-	-

Appendix B

Distance matrix showing genetic distances, computed using Kimura's 2 parameter model (Kimura 1980), between all pairs of individuals included in the phylogenetic analyses is shown on pages 113 to 116.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	Fox	-																									
2	Fox D83639	0.02	-																								
3	Coyote 14	0.15	0.16	-																							
4	Coyote 16	0.14	0.16	0.01	-																						
5	Arctic Wolf 1	0.16	0.17	0.06	0.06	-																					
6	European Wolf 1	0.16	0.18	0.06	0.06	0.00	-																				
7	European Wolf 2	0.16	0.17	0.06	0.06	0.00	0.01	-																			
8	Yukon Wolf 2	0.16	0.17	0.06	0.06	0.01	0.01	0.01	-																		
9	Yukon Wolf 4	0.15	0.17	0.06	0.06	0.01	0.01	0.01	0.01	-																	
10	Yukon Wolf 5	0.16	0.17	0.06	0.06	0.01	0.01	0.00	0.01	0.01	-																
11	Yukon Wolf 6	0.16	0.17	0.06	0.06	0.01	0.01	0.00	0.01	0.01	0.00	-															
12	Yukon Wolf 22	0.16	0.17	0.06	0.06	0.01	0.01	0.01	0.01	0.00	0.01	0.01	-														
13	Van Isld Wolf 1	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	-													
14	Van Isld Wolf 2	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.00	-												
15	Van Isld Wolf 3	0.16	0.17	0.06	0.06	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.01	0.02	0.02	-											
16	Tahltan 1	0.23	0.27	0.09	0.09	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.00	0.02	0.02	0.01	-										
17	Tahltan 2	0.23	0.26	0.09	0.09	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.00	0.01	0.01	0.01	0.00	-									
18	Hist Dog 41	0.23	0.26	0.09	0.09	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	-								
19	Village Dog 74	0.23	0.27	0.09	0.09	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	-							
20	Wool Dog 64	0.22	0.27	0.09	0.09	0.02	0.02	0.03	0.04	0.02	0.03	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	-						
21	GenBank Dog	0.22	0.25	0.08	0.08	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	-					
22	The Other Dog	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.00	0.02	0.01	0.01	0.01	0.02	0.02	0.00	-				
23	Akita 1	0.15	0.17	0.06	0.05	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	-			
24	Greyhound 2	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	-		
25	Nrw Elkhound 5	0.15	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.02	-	
26	Samoyed 7	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.01	0.00	0.02	-
27	Samoyed 8	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.01	0.00	0.02	0.00
28	S Husky 10	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.00
29	S Husky 11	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.01	0.01	0.01	0.02	0.01
30	Whippet 13	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
31	Whippet 15	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
32	Whippet 16	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
33	Gt Pyrenees 17	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.00	0.00	0.02	0.00
34	Whippet 18	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
35	Nrw Elkhound 19	0.15	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.02	0.02	0.00	0.02
36	Malamute 20	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
37	Malamute 22	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
38	Akita 23	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.00	0.01	0.00	0.03	0.00
39	Malamute 24	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.01	0.01	0.02	0.00
40	Shar Pei 25	0.16	0.18	0.05	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.00	0.01	0.01	0.02	0.00
41	Gt Pyrenees 27	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.00	0.00	0.02	0.00
42	Maremma 28	0.15	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
43	Maremma 31	0.15	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
44	Maremma 33	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.00	0.00	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.02	0.00
45	D83599	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.01

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
46	D83600	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.02	0.00
47	D83601	0.17	0.17	0.06	0.06	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.02
48	D83602	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.00	0.01	0.00	0.02	0.01
49	D83604	0.16	0.17	0.06	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.01	0.02
50	D83605	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
51	D83606	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.00	0.00	0.02	0.00
52	D83607	0.17	0.18	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
53	D83608	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01
54	D83609	0.16	0.17	0.06	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.00	0.01	0.01	0.02	0.00
55	D83610	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.00	0.00	0.00	0.02	0.00
56	D83611	0.17	0.18	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.04	0.04	0.03	0.02	0.01	0.01	0.01	0.03	0.01
57	D83612	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.00	0.00	0.00	0.03	0.00
58	D83613	0.17	0.18	0.06	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.00	0.01	0.01	0.02	0.00
59	D83616	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.01
60	D83618	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
61	D83619	0.16	0.17	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
62	D83620	0.17	0.17	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
63	D83621	0.17	0.18	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.01	0.00	0.01	0.01	0.02	0.01
64	D83622	0.17	0.17	0.07	0.07	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.02
65	D83623	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.00
66	D83624	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.02	0.03	0.03	0.00	0.03	0.01	0.02	0.01	0.02	0.02
67	D83625	0.17	0.18	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
68	D83626	0.16	0.17	0.05	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.02	0.00
69	D83627	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.02	0.00
70	D83628	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.00	0.00	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.00	0.01	0.00	0.02	0.01
71	D83629	0.17	0.18	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.03	0.01
72	D83631	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.01	0.01	0.00	0.02	0.02	0.01	0.02	0.03	0.02	0.02	0.01	0.01	0.01	0.02	0.01
73	D83632	0.16	0.17	0.06	0.06	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.03	0.02	0.01	0.02	0.02	0.02	0.02
74	D83633	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.00	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.00
75	D83634	0.17	0.18	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.03	0.02	0.02	0.03
76	D83635	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.01	0.00	0.02	0.01
77	D83636	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.02	0.02	0.03	0.00	0.03	0.01	0.02	0.01	0.02	0.02
78	D83637	0.17	0.18	0.06	0.06	0.02	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.04	0.03	0.04	0.04	0.04	0.02	0.01	0.02	0.02	0.03	0.01
79	D83638	0.16	0.17	0.06	0.06	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.02	0.03	0.03	0.00	0.03	0.01	0.02	0.01	0.02	0.02

		27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
27	Samoyed 8	-																									
28	S Husky 10	0.00	-																								
29	S Husky 11	0.01	0.01	-																							
30	Whippet 13	0.00	0.00	0.01	-																						
31	Whippet 15	0.00	0.00	0.01	0.00	-																					
32	Whippet 16	0.00	0.00	0.01	0.00	0.00	-																				
33	Gt Pyrenees 17	0.00	0.00	0.01	0.00	0.00	0.00	-																			
34	Whippet 18	0.01	0.00	0.01	0.00	0.00	0.00	0.01	-																		
35	Nrw Elkhound 19	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-																	
36	Malamute 20	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	-																
37	Malamute 22	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	-															
38	Akita 23	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.03	0.01	0.01	-														
39	Malamute 24	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	-													
40	Shar Pei 25	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	-												
41	Gt Pyrenees 27	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.02	0.01	0.01	0.00	0.01	0.01	-											
42	Maremma 28	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	-										
43	Maremma 31	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.00	-									
44	Maremma 33	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	-								
45	D83599	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	-							
46	D83600	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	-						
47	D83601	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-					
48	D83602	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	-				
49	D83604	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	-			
50	D83605	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.00	0.02	-		
51	D83606	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.02	0.01	-	
52	D83607	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.00	0.02	0.00	0.02	0.02	-
53	D83608	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.00	0.02	0.00	0.01	0.03
54	D83609	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.01	0.02	0.00	0.01	0.02
55	D83610	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.02	0.01	0.02	0.01	0.00	0.03
56	D83611	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.03	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.03
57	D83612	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.03	0.01	0.00	0.03
58	D83613	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.02	0.01	0.02	0.01	0.01	0.02
59	D83616	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.02	0.00	0.00	0.02
60	D83618	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.00	0.02	0.00	0.01	0.02
61	D83619	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.00	0.02	0.00	0.02	0.02	0.00
62	D83620	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.00	0.02	0.00	0.02	0.02	0.00
63	D83621	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.01	0.02	0.01	0.01	0.03
64	D83622	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.00	0.02	0.02	0.00
65	D83623	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.02	0.01	0.02	0.00	0.00	0.02
66	D83624	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02
67	D83625	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.00	0.02	0.00	0.02	0.02	0.00
68	D83626	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.03
69	D83627	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.02
70	D83628	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.00	0.02	0.00	0.01	0.02
71	D83629	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.03	0.01	0.01	0.03

		27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
72	D83631	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.00	0.02	0.00	0.01	0.03
73	D83632	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.02	0.01
74	D83633	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.00	0.02
75	D83634	0.03	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.00	0.02	0.00	0.02	0.02	0.00
76	D83635	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.01	0.02	0.01	0.02	0.01	0.01	0.03
77	D83636	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.02
78	D83637	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.01	0.03	0.02	0.03	0.02	0.02	0.03
79	D83638	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02

		53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78
53	D83608	-																									
54	D83609	0.01	-																								
55	D83610	0.01	0.00	-																							
56	D83611	0.02	0.01	0.01	-																						
57	D83612	0.01	0.00	0.01	0.02	-																					
58	D83613	0.01	0.01	0.00	0.01	0.00	-																				
59	D83616	0.00	0.01	0.01	0.02	0.01	0.01	-																			
60	D83618	0.00	0.01	0.01	0.02	0.01	0.01	0.00	-																		
61	D83619	0.03	0.02	0.03	0.03	0.03	0.02	0.02	0.02	-																	
62	D83620	0.03	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.00	-																
63	D83621	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.03	-															
64	D83622	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.00	0.00	0.02	-														
65	D83623	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.02	0.02	0.01	0.02	-													
66	D83624	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.01	-												
67	D83625	0.03	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.00	0.00	0.03	0.00	0.02	0.02	-											
68	D83626	0.01	0.00	0.01	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.02	0.00	0.02	0.03	-										
69	D83627	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.02	0.02	0.01	0.02	0.00	0.01	0.02	0.01	-									
70	D83628	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.02	0.02	0.01	0.02	0.01	0.01	0.02	0.01	0.00	-								
71	D83629	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.03	0.01	0.02	0.03	0.01	0.01	0.01	-							
72	D83631	0.01	0.01	0.01	0.02	0.01	0.01	0.00	0.00	0.03	0.03	0.01	0.02	0.01	0.02	0.03	0.01	0.01	0.00	0.01	-						
73	D83632	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	-					
74	D83633	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.02	0.02	0.01	0.02	0.00	0.01	0.02	0.01	0.00	0.00	0.01	0.00	0.02	-				
75	D83634	0.02	0.02	0.03	0.03	0.03	0.02	0.02	0.02	0.00	0.00	0.03	0.00	0.02	0.02	0.00	0.03	0.02	0.02	0.03	0.03	0.01	0.02	-			
76	D83635	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.02	0.01	0.02	0.03	0.01	0.00	0.01	0.00	0.01	0.02	0.00	0.03	-		
77	D83636	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.00	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02	-	
78	D83637	0.02	0.02	0.01	0.00	0.02	0.01	0.02	0.02	0.03	0.03	0.01	0.03	0.01	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	-
79	D83638	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.00	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.00	0.02

		79
79	D83638	-

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Burbidge, M.L., Byun, S.A., Crockford, S., Rink, U., Wiggen, R. and Koop, B.F. 1997. Dogs, mitochondrial DNA and ancient DNA techniques. Annual Meeting: **Society for Conservation Biology**, Victoria, British Columbia.

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