Chapter 5
Systematics, Population Genetics and Genetic Management of the Ethiopian Wolf

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Introduction

Molecular genetic techniques can be used to address several questions of interest to conservation biologists. Significantly, recent advances in DNA technology have allowed a finer precision to investigations. DNA techniques can now potentially be used to identify parents, offspring, and close relatives in a single group or population, to quantify the genetic variability of present and past populations, to reconstruct the phylogenetic relationships of taxa now very rare or extinct and to match samples of individuals to each other and to species or populations for forensic purposes. Moreover, the quantity of material required for DNA analyses may be minute, for example, single hairs, serum, or archaeological or museum samples of pelts and bone. Because of the recency of these techniques, they have yet to be widely applied to significant problems involving the management and conservation of small populations. In this review, we will address several genetic issues important to the conservation of Ethiopian wolves (*Canis simensis*) that span ever finer levels of evolutionary divergence. At the highest level are questions about the uniqueness of species relative to other species within the same genus or higher taxonomic unit, and at the lowest level, questions concerning the parentage and reproductive success of individuals.

Systematics and Phylogenetic Distinction

The phylogenetic position of the Ethiopian wolf is uncertain as some researchers have suggested relationship to African jackals rather than wolves (see Sillero-Zubiri and Gottelli 1994). In general, the systematics and phylogenetic distinction of endangered species is an important concern for conservation biologists (*e.g.* May 1990). Phylogenetic analysis based on morphological or molecular methods allows a ranking of species on a scale reflecting the distinctiveness of their evolutionary heritage. For example, we might consider a giant panda (*Ailuropoda melanoleuca*) more distinct in a phylogenetic sense relative to other bears, because it is the only living representative of an entire subfamily. In contrast, a single genus of beetle may encompass hundreds of genetically similar species. Therefore, because each species of such beetles contains less distinct genetic information than would the sole representative of a higher taxon, it might be accorded a lower priority for conservation measures. Of course, other characteristics such as overall endangerment, the role in the ecosystem of each species and their value as flagship species, need to be considered as well. Comprehensive discussion of molecular techniques as applied to systematic questions may be found in Hillis and Moritz (1990) and discussion of schemes which rank species according to their position in a phylogenetic network are discussed by Vane-Wright *et al.* (1990) and Faith (1994).

Hybridization

An important genetic concern in Ethiopian wolves is the degree of interbreeding with domestic dogs (Sillero-Zubiri *et al.* 1993, Chapter 3). Interbreeding among individuals from closely related species and among individuals from distinct populations of the same species is a common natural phenomenon (Barton and Hewitt 1985, 1989, O'Brien and Mayr 1991). However, in the Ethiopian wolf, interbreeding is not a natural phenomenon but is due to the recent introduction of domestic dogs. Human activities have led to hybridization between other species, for example coyotes (*Canis latrans*), grey wolves (*C. lupus*) and domestic dogs in North America and Europe (Lehman *et al.* 1991, Wayne and Jenks 1991), the Florida puma (*Felis concolor coryi*) and a non-native subspecies of puma (O'Brien *et al.* 1990), and native fish populations and hatchery raised fish (Waples and Teel 1990). Similarly, captive breeding stocks may often represent a blend of subspecies through unintentional interbreeding. An example is the Asiatic lion (*Panthera* *leo persica*), for which breeding stocks at many zoos have been found to contain genes from the African subspecies (O'Brien *et al.* 1987). This discovery caused a dramatic change in the captive breeding program of the Asiatic lion. Genetic screening of wild, endangered populations or captive stocks suspected of such artificial hybridization may provide essential data for on-site conservation or captive breeding programs.

Population Genetic Units

The Ethiopian wolf had a historic distribution throughout the Ethiopian highlands and given the presence of topographic and environmental barriers to dispersal, some populations may have acquired important genetic differences that should be preserved by captive breeding and *in situ* genetic management. Two subspecies are currently recognized, one from either side of the Ethiopian Rift Valley, based on craniological differences (Yalden *et al.* 1980, Chapter 1). Molecular genetic analysis can be used to document the genetic distinctiveness of subspecies and other population genetic units that existed within species. In addition, molecular genetic techniques can be used to trace corridors of dispersal among populations and to identify populations that might provide the source material for re-stocking or reintroduction programs. Re-stocking of an endangered population may be necessary if inbreeding has significantly affected viability. Given this condition, re-stocking with individuals from a similar environment and with a similar genetic constitution to that of the source population may be desirable.

Genetic Variability

The small population size of Ethiopian wolves suggests that genetic variability may be limited and will continue to decline unless numbers increase. Genetic variability generally comprises two components, allelic diversity (or the number of alleles at a given locus), and genetic heterozygosity (or the expected proportion of genes in the heterozygous state in the average individual). In small populations, genetic variability may be rapidly reduced; initially allelic diversity decreases followed by reduced heterozygosity levels (Allendorf 1986). Such decreases in heterozygosity, especially in association with breeding among close relatives, may correspond with decreases in viability and increased juvenile mortality (Allendorf and Leary 1986, Ralls and Ballou 1983, Ralls *et al.* 1988, Quattro and Vrijenhoek 1989). Genetic variability is thought to be essential to the long-term persistence and adaptability of populations and thus management of captive and wild populations of endangered species should be designed to minimize the loss of genetic variability. Both morphologic and molecular techniques can be used to compare variability among populations and to follow the decline of variability in small populations (*e.g.* Wayne *et al.* 1991a, 1991b). The discovery of hyper-variable mini- and micro-satellite loci may potentially increase the sensitivity of genetic variability measurements (*e.g.* Taylor *et al.* 1994).

Genetic Management

Finally, there are several genetic questions important to the establishment and genetic maintenance of captive populations of Ethiopian wolves and to their reintroduction or the genetic augmentation of populations in the wild. In wild and captive populations, there may be great asymmetries in reproductive success among individuals and sexes such that the effective population size is reduced and breeding among close relatives occurs. To reduce the loss of genetic variability in small populations, the genetic relationships of individuals and breeding structure need to be understood so that the number of breeders and their genetic dissimilarity is maximized. Moreover, the founders of a captive population should be chosen such that they are unrelated and best represent the genetic diversity within the source population. Animals of hybrid ancestry should also be excluded if hybridization is not part of the evolutionary history of the population (*e.g.* De Marais *et al.* 1992). Molecular genetic techniques can effectively be used to deduce parentage in wild and captive populations, to identify individuals that are close relatives, and that have population specific polymorphisms (Avise 1994). Even in populations for which little information is available, inferences can be made about the breeding structure from molecular genetic data (*e.g.* Packer *et al.* 1991, Lehman *et al.* 1992).

Molecular-genetic Techniques

Within species, canids generally show only low levels of allozyme polymorphism (*e.g.* Wayne *et al.* 1991*b*, Kennedy *et al.* 1991). Consequently, population genetic studies focused on the mitochondrial genome because in mammals its sequence generally evolves much faster than most nuclear genes (Brown 1986). Moreover, because the mitochondrial genome is maternally inherited in a clonal fashion without recombination, analysis of mitochondrial DNA (mtDNA) sequences in populations provides a history of maternal lineages that avoids the reticulation caused by recombination and may allow for a precise reconstruction of colonization events, gene flow and hybridization (Avise 1994). Initially, we characterized mtDNA variation using a restriction fragment analysis approach (*e.g.* Lehman *et al.* 1991, Wayne *et al.* 1992). More recently, with the advent of the polymerase chain reaction and the identification of universal primers, we have begun to compare directly hyper-variable mitochondrial sequences in large population samples (Wayne and Jenks 1991; Girman *et al.* 1993; Mercure *et al.* 1993; Maldonado *et al.* 1995). Such techniques can be used on samples obtained through non-invasive sampling, which include samples of hair and faeces (*e.g.* Hoss *et al.* 1992; Morin *et al.* 1994).

However, mitochondrial (mtDNA) analysis provides only one perspective on genetic variation. Levels of mtDNA variation are more severely affected than nuclear loci by changes in population size and phylogenetic trees based on mtDNA sequence data record the history of only a single linked set of genes. Formerly nuclear genes with equivalent evolutionary rates had not been identified or were difficult to survey in large population samples. Recently, hyper-variable nuclear loci have been characterized in a diverse array of taxa that are composed of tandem repeats of very short sequences, 2–5 base pairs (bp) in length. These simple sequence repeat or micro-satellite loci can easily be amplified by the polymerase chain reaction and separated on acrylamide gels. This procedure allows an assay of individual loci that are highly polymorphic in large population samples and, as above, allows the use of degraded material including bones, hair and faecal material (Hagelberg *et al.* 1991; Hoss *et al.* 1992; Roy *et al.* 1994*a*; Morin *et al.* 1994). A panel of only a dozen or fewer micro-satellite loci may be sufficient to quantify components of variation within and among populations and to study individual relatedness within social groups (*e.g.* Amos *et al.* 1993; Roy *et al.* 1994*b*). We have assessed variation for about 10 highly polymorphic micro-satellite loci in several canid species and find they provide an extremely useful contrast to past surveys of mtDNA variability (see below).

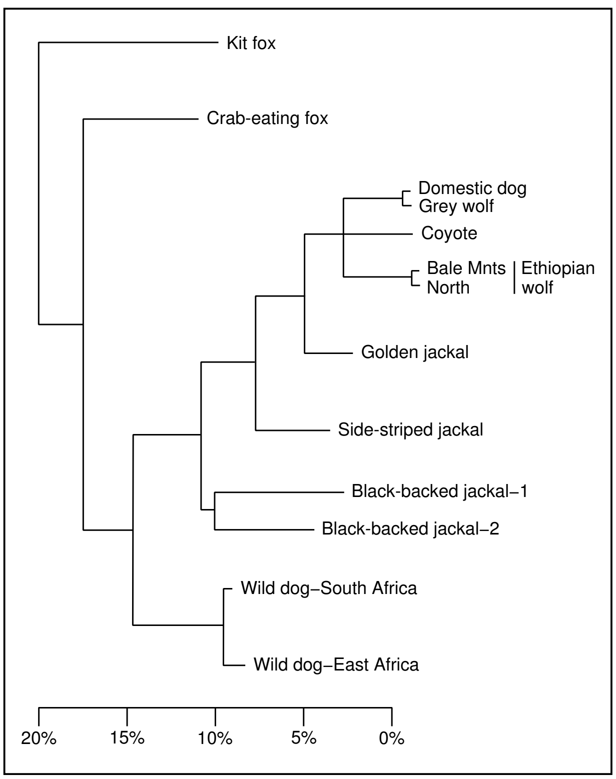


Figure 5.1. A strict consensus tree of the two most parsimonious trees obtained from phylogenetic analysis of 2001 base pairs of mitochondrial DNA sequence (Gottelli et al. 1994).
Two individuals were sequenced for each species. The two east African black-backed jackal sequences (1 and 2) are representatives of the two divergence mtDNA genotypes found there (Wayne et al. 1990). The grey fox sequence was used to root the tree. Nodes supported in over 50% of 1000 bootstrap trees are indicated.

Results and Discussion

Systematics and Phylogenetic Distinction – a Wolf in Africa

Our phylogenetic analysis of the sequence of 2001 base pairs of mtDNA showed conclusively that the Ethiopian wolf is a close relative of grey wolves, domestic dogs, and coyotes (Fig. 5.1, Gottelli *et al.* 1994). This association was our motivation to use Ethiopian wolf as a common name instead of the more frequently used Simien jackal or Simien fox (Chapter 1). The phylogenetic tree provides an evolutionary yardstick to measure the distinction of Ethiopian wolves; they are a distinct species, as different from grey wolves and coyotes as each of these species is from each other. As the only close relative of grey wolves and coyotes in Africa, they are clearly a unique taxon worthy of conservation and are phylogenetically distinct from the African wild dog (*Lycaon pictus*) and the three species of African jackals.

The phylogenetic relationships of Ethiopian wolves and their restriction to afroalpine grasslands above 3,000 m suggests an unusual history. We hypothesized that Ethiopian wolves are a relict species resulting from a Pleistocene invasion of a wolf-like canid into the once more extensive Afroalpine ecosystem. This species has become remarkably well adapted to existence in the high altitude grasslands and heathlands. Its social behaviour, feeding ecology and morphology differ from other wolf-like canids (Chapter 1) and contrast with those of the savanna-living, African wild dog. The unique attributes of the Ethiopian wolf and its distinct evolutionary history, highlight the urgent need for its conservation.

Population Genetic Units

The two populations we studied in detail from the Bale Mountains National Park were located in the Sanetti Plateau and the Web Valley, two areas separated by 20 kilometres of inhospitable rocky peaks, crossed by narrow corridors of suitable habitat (see Fig. 2.6). We found that both populations had the same mtDNA sequence. More significantly, micro-satellite allele frequencies differed little between the two populations. A measure of population differentiation, F_{st}, was 0.057 indicating that only about 6% of the genetic variation was distributed between populations. The Nei's genetic distance between the two populations is only 0.025 which contrasts with the average value of 0.55 between them and grey wolves or 0.47 between them and domestic dogs. Migration rates based on this value of F_{st} are large, about 4.3 migrations per generation. Because, more than two migrants per generation are sufficient to confound genetic divergence due to drift in finite populations (Slatkin 1987), and given the absence of genetic distinctiveness, the two localities may be considered as not genetically isolated.

The molecular techniques we used can also be applied to museum pelt specimens. We analyzed a small highly variable segment of the mtDNA control region in two museum skins from an extinct northern population (Fig. 2.2, Roy *et al.* 1994a). We found these skin samples had identical sequences but that both were different in two of 134 base pairs from wolves in the Bale Mountains to the South. This amount of sequence divergence (about 1.5%) is relatively small for this hyper-variable region of mtDNA (called the control region), and may be the effect of recent isolation since the last glaciation 10,000–70,000 years ago. In contrast, Ethiopian wolves differ from domestic dogs and grey wolves by about 11% of the control region sequence. Consequently, although Northern populations may be genetically isolated from those in the Bale Mountains, the amount of genetic differentiation is minimal compared to that which exists between species or long-isolated subspecies (*e.g.* Wenink *et al.* 1993; Maldonado *et al.* 1995). If the population of Ethiopian wolves from the North was large, separate captive breeding plans might be considered. However, because most populations from the North are extinct, large number of individuals cannot be obtained from either northern or southern locations to establish captive breeding populations. We suggest that concern for preserving this limited degree of population differentiation should not override concerns about inbreeding depression in small separately maintained captive populations.

Genetic Variability

The demographic history of Ethiopian wolves is marked by both long term and recent range reductions. The current limited fragmented distribution of the Ethiopian high altitude afroalpine habitats is only 5% of the area existing after the last Ice Age (see Gottelli *et al.* 1994). Consequently, the geographic range and numerical abundance of Ethiopian wolves has been progressively decreasing during the Holocene. Recently, a more rapid decline in habitat has occurred as habitat loss and fragmentation was accelerated by human population growth and agriculture (Gottelli and Sillero-Zubiri 1992, Chapter 2).

Mitochondrial DNA sequence and restriction site analysis showed that the two populations that we surveyed in the Bale Mountains had a single mitochondrial genotype, the most limited population variability of any extant canid, except those populations isolated on islands (Table 5.1, Gottelli et al. 1994). Similarly, average variability of micro-satellite loci was also dramatically reduced to 46% of the heterozygosity and 38% of the allelic diversity of an average population of wolf-like canids (Table 5.1). All of the genotype distributions fit Hardy-Weinberg expectations as determined by chi-square tests ($p < 0.05$). Overall heterozygosity and allelic diversity appeared lower on the Sanetti Plateau; only six of nine loci were polymorphic and average levels of heterozygosity were significantly lower (Fig. 5.2, Gottelli et al. 1994). In general, such low values of heterozygosity are consistent with an equilibrium effective population size of only a few hundred individuals. Moreover, recent habitat fragmentation has likely decreased the effective population size to a value much lower than this. Consequently, levels of genetic variation will rapidly decline in both populations, especially in more slowly evolving loci of the kind that might influence continued adaptive change within the population. In fact, recent theoretical analysis suggests that at least five thousand individuals are needed in order to prevent drift from fixing mildly deleterious genes, and causing the population to be less fit (Lande 1995). Similarly, in the other still remaining isolated populations of Ethiopian wolves, loss of genetic variation and inbreeding will occur, increasing the immediate probability of population extinction. In sum, our results show that Ethiopian wolves have already lost substantial genetic variation. This loss will accelerate given the present extent of habitat fragmentation and will be a negative influence on the survival of populations in the short and long term.

Hybridization

The close relationship of Ethiopian wolves to domestic dogs suggests that they may be able to hybridize with them just as dogs, coyotes and grey wolves hybridize with each other (Gray 1954, Lehman *et al.* 1991). Although as discussed above, loss of variation and inbreeding is a concern, a more pressing issue with Ethiopian wolves is hybridization with domestic dogs. Domestic dogs are abundant in the Web Valley and are often only loosely associated with humans (Chapter 3). In contrast, domestic dogs are nearly absent from the Sanetti Plateau. Field researchers observed that many Ethiopian wolves in Web Valley had unusual coat coloration and morphology and suspected hybridization because of local reports of dogs mating with wolves

Table 5.1
Number of mtDNA restriction site genotypes, maximum percent mtDNA sequence divergence within a species, and the mean number of microsatellite alleles per locus and their mean heterozygosity for ten canid species. References for these data are indicated.

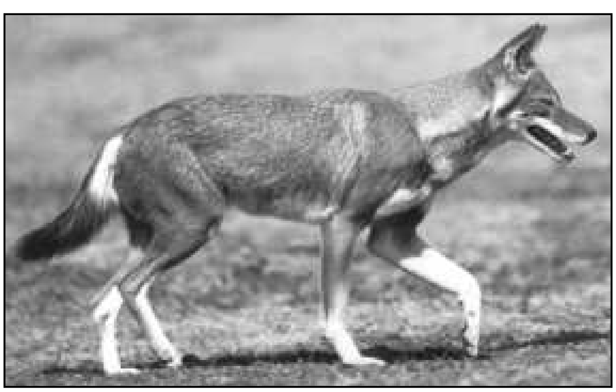
Species	Number of genotypes	% Sequence divergence	Alleles per locus	Heterozygosity	Reference
Ethiopian wolf *(Canis simensis)*	1	0.0	2.4	0.24	a
Black-backed jackal *(Canis mesomelas)*	4	8.4	5.0	0.67	b
Golden jackal *(Canis aureus)*	2	0.1	4.8	0.52	b
Side-striped jackal *(Canis adustus)*	2	0.2	ND	ND	b
Coyote *(Canis latrans)*	32	2.5	5.9	0.68	c
Gray wolf *(Canis lupus)*	9	0.8	4.5	0.62	d
Kit fox *(Vulpes macrotis)*	24	1.5	ND	ND	e
Channel Island fox *(Urocyon littoralis)*	5	1.8	2.1	0.24	f
African wild dog *(Lycaon pictus)*	6	0.9	3.5	0.56	g

a) Gottelli *et al.* 1994; b) Wayne *et al.* 1990; c) Lehman and Wayne 1991, Roy *et al.* 1994a; d) Wayne *et al.* 1992, Roy *et al.* 1994a; d) Mercure *et al.* 1993; e) Geffen *et al.* 1992; f) Wayne *et al.* 1991a, Wayne *et al.* 1995; h) Girman *et al.* 1993.

(Sillero-Zubiri *et al.* 1993). Our initial mtDNA analysis showed suspected hybrid individuals to have genotypes identical to those in '*pure*' Ethiopian wolves from Sanetti. However, based on observation of dog-wolf interactions and the Ethiopian wolf mating system (Chapter 3, Sillero-Zubiri *et al.* 1996b) interspecific matings would be expected between male domestic dogs and female wolves, and not the other way round. Therefore an Ethiopian wolf mitochondrial genotype would be expected in dog-wolf hybrids because of the maternal inheritance of mitochondrial DNA. Consequently, we analyzed pure wolves and suspected hybrids for variation in 10 micro-satellite loci. This analysis showed that all the suspected hybrid wolves had one or more diagnostic dog marker alleles (*e.g.* Fig. 5.2). The presence of several dog marker alleles in phenotypically abnormal wolves confirmed that hybridization was occurring and provided an important justification for dog control. Sympatric dogs threaten Ethiopian wolves in other ways as well; they not only hybridize with them, but are reservoirs of canine diseases and compete with them for food (see Chapter 4).

Patterns of Reproduction within Populations

We had limited data on reproduction patterns in Ethiopian wolves. In one pack, we found that the alpha female had bred with at least two males, neither of which was the alpha male in the pack. One of these males must have carried dog alleles and was likely a dog-wolf hybrid or a domestic dog. This instance of multiple paternity is the first confirmed for a wild canid. In another pack offspring of the alpha female also had dog alleles not found in the alpha male. However, multiple paternity was not necessarily occurring because a single paternal genotype could be constructed that would satisfy the distribution of alleles in this litter. These very preliminary results suggests a more open reproductive system than exists in grey wolf packs which are thought to consist of a monogamous pair and their offspring (Mech 1970). The genetic results indicate that female Ethiopian wolves have multiple mates that may not be an established member of their pack supporting direct observation of mating patterns that report 70% of all matings (n = 70) taking place with extra-pack males (Sillero-Zubiri *et al.* 1996b). This open reproductive system may make the threat of hybridization with roaming dogs more severe, and suggests that captive breeding schemes should allow for multiple matings with males outside the pack.



Genetic Management

No Ethiopian wolves are kept or bred in zoos. A great concern for the survival of Ethiopian wolves is the vulnerability of the remaining populations to stochastic demographic effects and to increased inbreeding and loss of genetic variability. Rabies is thought to have eliminated about half of the Bale Mountains population since 1990 (Sillero-Zubiri *et al.* 1996a) and human persecution and further agricultural development threaten all populations (see Chapters 2–4). Thus, in addition to immediately halting the decline of wild wolves there is an urgent need to establish a captive population as a hedge against further cataclysmic population declines. Unfortunately, progress in establishing a captive population has been slow. The primary difficulty has been obtaining permission to capture and breed individuals from the wild, the construction of breeding facilities in Ethiopia and other prerequisites for exporting wolves to breeding facilities outside the country.

Given these problems can be solved, several genetic considerations need to be addressed. A first concern is the number and selection of individuals from the wild. Clearly, if wolves were more abundant, a large founding stock of 50 or more individuals would be desirable to represent and preserve the diversity of the wild population (*e.g.* Lacy 1987). However, captive populations have been founded with fewer individuals and succeeded although some inbreeding depression may occur. A living model is provided by the captive programme for the red wolf (*Canis rufus*), where only 14 individuals were selected from the wild but rapid expansion of the captive population and careful genetic management keep inbreeding to a minimum. Our own analysis of the red wolf population has shown them to have high levels of allelic diversity and heterozygosity (Roy *et al.* 1994b). The important concern here is that the founder population should be managed to reduce inbreeding and the loss of variability and should be expanded rapidly. To accomplish this, computer-based pedigree management is needed (*e.g.* large scale breeding facilities should be considered that involves the cooperation of many zoos. For the red wolf, a breeding facility was constructed near Seattle that can accommodate about 200 wolves and in addition over a dozen zoos participate in the programme.

Having secured permission to capture 14 or more individuals for captive breeding, they should be selected to represent the genetic diversity of wild populations before the introduction of domestic dogs.

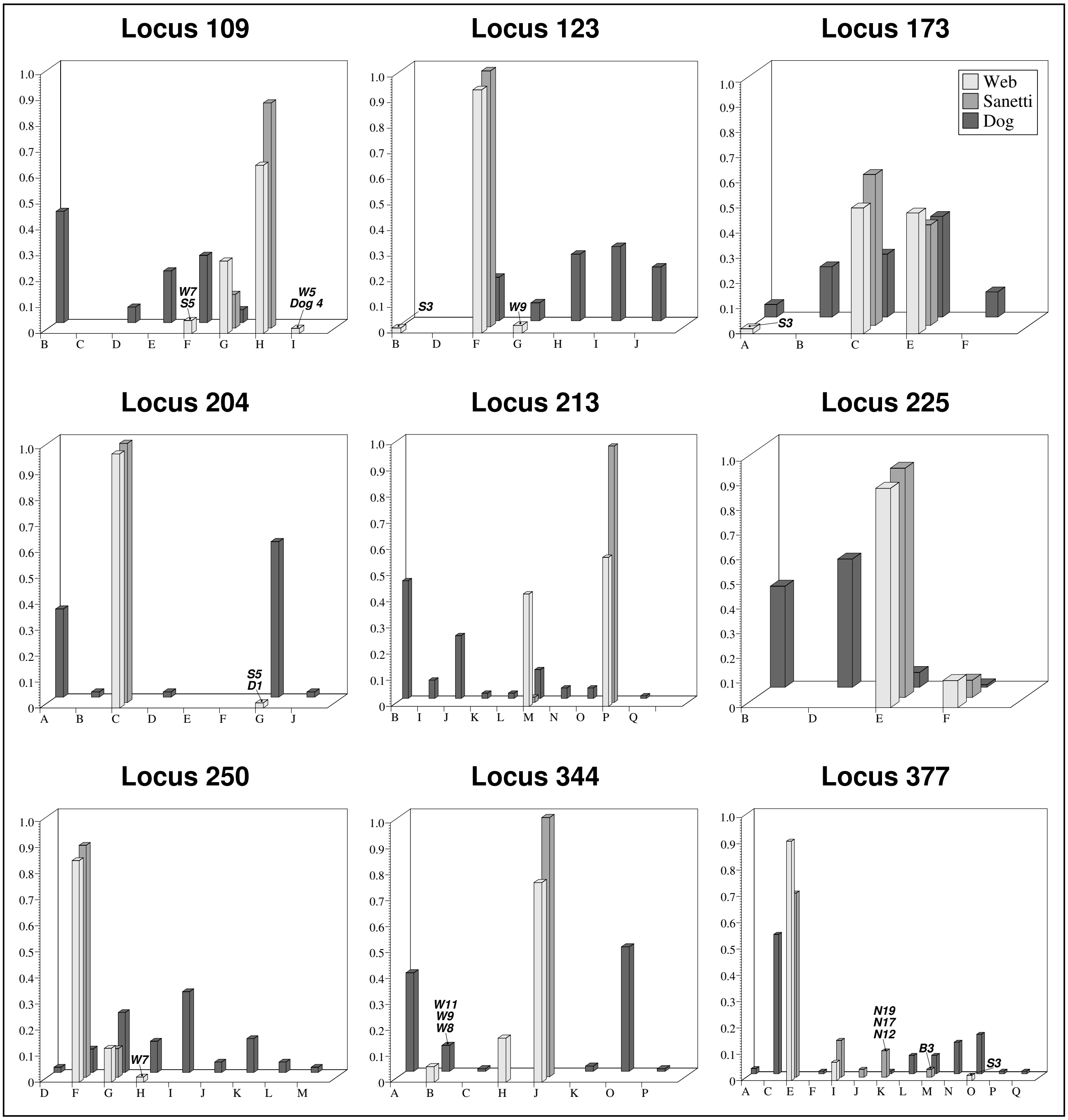


Figure 5.2. Allele frequency histograms for nine microsatellite loci in Ethiopian wolves from the Web Valley and Sanetti and in a sample of 32 domestic dogs.
Consecutive letters differ by a single two base pair repeat unit. Ethiopian wolves that were suspected to be hybrids based on phenotypic criteria are indicated by their ID. All suspected hybrids have one or more alleles that are otherwise found in domestic dogs but absent from Sanetti wolves where domestic dogs are not common.

This might involve the selection of individuals from different packs that appear phenotypically normal and have them tested using genetic methods that might detect dog ancestry. Moreover, the genetic relatedness between potential founders can be estimated using molecular techniques and the selection of closely related individuals avoided. Finally, if multiple paternity is common in the wild, captive breeding plans may wish to consider multiple insemination of females by different males to maximise the chance that they may become pregnant and at the same time allow genetic contributions from an increased number of males to be included in the next generation. This genetic strategy may also better mimic the pattern that occurs in the wild.

Finally, an urgent need is the development of specific protocols for the collection of viable sperm and eggs from wild wolves that could be frozen for future use in captive breeding. It is now possible in some exotic carnivores to collect sperm through electro-ejaculation of wild males who are then released and use the sperm as a source for artificial insemination or to fertilize harvested eggs in vitro (*e.g.* Donoghue *et al.* 1992a, 1992b). Similarly, eggs could potentially be flushed from wild caught females, viably frozen and matured at later date for fertilization and implantation in captive wolves (*e.g.* Johnston *et al.* 1991, 1994). Such fertilized eggs could potentially be brought to term in surrogate mothers from a related species, such as domestic dogs. A genetic bank of eggs and sperm from wild caught wolves could be used to enrich the genetic diversity of captive wolves once the breeding program is started and in the meantime, would provide a hedge against the very real possibility of dramatic population declines and even extinction in the wild.

Summary

The Ethiopian wolf is a phylogenetically distinct African canid endemic to the Ethiopian highlands that is closely related to grey wolves, domestic dogs and coyotes. The species has a more distant relationship to African jackals. A primary threat to the persistence of the Ethiopian wolf is the presence of domestic dogs who hybridize with them, compete with them for food and act a reservoir of canine diseases. Hybridization between Ethiopian wolves and domestic dogs is widespread in the Web Valley. Hybrid offspring are incorporated into Ethiopian wolf packs and with the continued persistence of dogs in this population, and their spread to others as well, the species could be threatened with genetic extinction. The population of domestic dogs co-existing with Ethiopian wolves needs to be immediately reduced and better controlled. The genetic variability of the Ethiopian wolf is lower than that of any other wolf-like canid and will continue to decline unless measures are taken to stabilize wild populations. A captive breeding program should be initiated immediately in Ethiopia with genetically certified Ethiopian wolves. A captive population would provide a reserve in the event of a cataclysmic decline in the wild population and a source for reintroduction to areas where the species has gone extinct.