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ALLOZYME DIVERGENCE WITHIN THE CANIDAE

ROBERT K. WAYNE[1] AND STEPHEN J. O'BRIEN

Laboratory of Viral Carcinogenesis, Section of Genetics, National Cancer Institute, Frederick, Maryland 21701

Abstract.—Protein products of 51 genetic loci were analyzed by gel electrophoresis using extracts of blood and tissue culture specimens from 12 of the 14 extant canid genera. Genetic distances were calculated and used to derive phenetic trees. The results suggest that the Canidae can be divided into several distinct groups. The wolf-like canids are a group that includes species in the genus *Canis* and *Lycaon pictus* (African wild dog). *Speothos venaticus* (Brazilian bush dog) is weakly associated with this group. Based on the calibration of a consensus tree with a fossil date, *Canis mesomelas* (black-backed jackal) and *Speothos venaticus* separated first, approximately 6 million years before present (MYBP). *Lycaon pictus* and *C. latrans* (coyote) separated from the line leading to *C. lupus* (grey wolf) and *C. familiaris* (domestic dog) approximately 3 MYBP. These results suggest that the blade-like trenchant heel on the carnassial tooth has evolved independently at least twice within the Canidae.

Several distinct genetic stocks appear to have led to the extant South American canids. *Chrysocyon brachyurus* (maned wolf) is estimated to have diverged from *Dusicyon vetulus* (hoary fox) and *Cerdocyon thous* (crab-eating fox) approximately 6 MYBP. The divergence time of the last two genera is fairly recent (2–3 MYBP) and is coincident with the opening of the Panamanian land bridge. The remaining South American canid included in this survey, *Speothos venaticus*, is clustered with the wolf-like canids. The *Vulpes*-like canids are a distinct phenetic group that includes species in the genera *Vulpes, Alopex* and *Fennecus*. Their estimated time of divergence from all the other canids, approximately 9 MYBP, is among the oldest within the Canidae. Among the *Vulpes*-like canids we surveyed, *Alopex lagopus* (arctic fox) and *Vulpes macrotis* (kit fox) appear genetically most closely related. Finally, the biochemical data support the generic status of three canid genera: *Urocyon, Nyctereutes,* and *Otocyon*. These taxa are not closely related to any of the surveyed canid species. [Allozyme; electrophoresis; phenogram; Canidae; evolution; trenchant heel; South America.]

The Canidae is a morphologically diverse family of dog-like carnivores that, according to Stains (1975), includes 14 extant genera and 34 species (excluding *Dasycyon hagenbecki*, which is known from only one museum study skin). Classifications of the family have often conflicted, probably because of morphologic convergences (Huxley, 1880; Simpson, 1945; Langguth, 1969, 1975; Clutton-Brock et al., 1976; Van Gelder, 1978). In this study, we use a genetic approach, gel electrophoresis of soluble blood proteins, to analyze relationships of canids. Except for the rare Asiatic dhole, *Cuon alpinus*, and the possibly extinct *Atelocynus microtis* (short-eared dog), all genera of extant canids are represented in our sample.

Phenetic trees based on differences in gene frequencies may suggest instances of apparent rapid morphologic evolution and evolutionary parallelism (cf. Larson, 1984; Shaffer, 1984; Wake and Yanev, 1986). A potential instance of evolutionary parallelism among canids is the evolution of a trenchant or blade-like heel on the carnassial or meat-slicing teeth (M_1P^4) of carnivores. In most canids, the carnassial tooth has a bladed anterior portion and a posterior semi-circular basin. In canids with a trenchant heel, the basin is reduced and altered to form a second blade. Presumably, this increases the functional length of the carnassial blade and hence the ability to slice meat (Ewer, 1973; Van Valkenburgh, in press). Canids with this type of dentition are also characterized by a reduction of the post-carnassial molars whose function is primarily to grind bone and coarse plant foods. The presence of the trenchant heel in three canid species, *Speothos venaticus* (Brazilian bush dog); *Lycaon*

[1] Present address: Department of Biology, University of California at Los Angeles, Los Angeles, California 90024.

pictus (African wild dog), and *Cuon alpinus* (Asiatic dhole) led Simpson (1945) and Stains (1975) to unite them in a single subfamily, the Simoncyoninae. However, the latter two taxa are wolf-like in external body form and quantitative measurements of the cranial and appendicular skeleton and chromosome morphology tend to align *Lycaon* and *Cuon* with the wolf-like canids of the genus *Canis* (Chiarelli, 1975; Clutton-Brock et al., 1976; Dutrillaux, 1986; Wayne, 1986a, b; Wayne et al., 1987a). Moreover, *Lycaon* and *Cuon* appear to be ecological surrogates of the Holarctic species, *Canis lupus* (grey wolf), in Africa and Asia, respectively. Like the wolf, they are highly social hunters of large game (Nowak and Paradiso, 1983). In contrast, the South American trenchant heel dog, *Speothos venaticus* is small (<10 kg) and is proportioned more like a relatively slow, ambush hunter than a gracile wolf (Van Valkenburgh, 1985, 1987). Thus, the trenchant heel may have evolved in parallel in the two wolf-like canids and in *Speothos venaticus*. To test this idea, we analyze proteins from two genera of trenchant heel dogs: *Speothos* and *Lycaon*. Specimens of *Cuon* are extremely rare and were not available for analysis.

A second question concerns the rate and direction of morphologic evolution. A major evolutionary experiment was initiated by the closing of the Panamanian isthmus 2–3 million years ago (Marshall et al., 1982). Prior to this time, there were no placental terrestrial carnivores in South America. Into this largely depauperate carnivore fauna, ancestors of the recent South American canids entered and diversified. The result is an entirely endemic canid fauna of 10 recent species that are placed into 3–6 genera (Langguth, 1969, 1975; Clutton-Brock et al., 1976; Van Gelder, 1978). Relative to other canids, several species are morphologically atypical. For example, *Speothos* has a carnivorous dental formula combined with a short-legged and elongate body form. In contrast, *Chrysocyon brachyurus*, the maned wolf, or "fox-on-stilts", is extremely long-legged, a feature which presumably represents an adaptation to the long grass of the South American plains (Langguth, 1975). Most of the other South American canids can be described as fox-like, but vary considerably in size and morphology (Langguth, 1969). Forms directly ancestral to these diverse South American taxa are not known from the North American fossil record (Berta, 1979, 1984; Kurten and Anderson, 1980). Thus, a crucial question concerns whether such morphologic extremes could have evolved rapidly from a single ancestor that entered South America during the early Pliocene or whether several ancestral stocks gave rise to the extant South American species. In this study, the genetic relationship between two of the most unusual taxa, *Speothos* and *Chrysocyon*, as well as species from two other genera, *Cerdocyon* and *Dusicyon* are analyzed to determine if they form a closely-related and possibly monophyletic group.

Finally, relationships of canids based on our data are compared with those derived from morphologic and karyologic approaches (Langguth, 1969, 1975; Clutton-Brock et al., 1976; Van Gelder, 1978; Wurster-Hill and Centerwall, 1982; Berta, 1984; Wayne et al., 1987a, b). We compare available data from the fossil record with the branching patterns and divergence times indicated by the biochemical data.

MATERIALS AND METHODS

Products of 51 presumptive genetic loci were examined in 17 canid species (see Table 1 and Appendix 1), each represented by a single individual. Not all loci were scored in every species (see Appendices 1 and 2). With most species, larger sample sizes are extremely difficult to obtain due to the rarity of the species. The use of such a small sample size appears acceptable if the number of loci is sufficiently large (>30), the genetic distances between taxa are large (>0.17), and heterozygosity is low (<0.10) (Sarich, 1977; Nei, 1978; Gorman and Renzi, 1979; Nei et al., 1983). Because genetic distances between the canid genera were fairly large (Tables 2, 3) and the genic heterozygosity of the canids that have

been surveyed is generally low (Fisher et al., 1976; Simonsen, 1976) the use of a single individual to represent each species is justifiable.

Fifteen to 20 cc of whole blood in heparin were obtained from each of the canids listed in Table 1. Blood was then separated by centrifugation into components containing plasma, erythrocytes and leukocytes. The clear plasma and an aliquot of 1–2 cc of blood from the bottom of the tube are removed (leaving the interface with white blood cells intact). The red cell aliquot is washed two times in buffered saline. The remaining blood is lysed with two volumes ACK lysing buffer for approximately 10 minutes, pelleted and washed in buffered saline. Red and white cells are prepared for electrophoresis by sonication and three cycles of freeze-thawing to release soluble blood proteins into the supernatant. After centrifugation, the supernatant was stored at −70°C. We obtained skin biopsies of most of the canids and used these to establish primary fibroblast cultures (see Table 1). Fibroblast cultures from canids grew slowly so fibroblast lines were transformed with a feline retrovirus to obtain rapid cell proliferation (Wayne et al., 1987a). Tissue obtained from these transformed cell lines was prepared for electrophoresis by washing twice in buffered saline followed by three freeze-thaw cycles as outlined above.

Electrophoresis of the 51 protein products was performed according to the conditions given in Appendix 1. Depending on the tissue specificity of each enzyme and the availability of samples, each locus was assayed in as many tissues as possible (Table 1, Appendix 1). Allozyme polymorphisms can be more confidently scored using this approach because their presence can be corroborated in different tissues. Allozyme polymorphisms were given alphabetical designations with the most common allele labeled A.

Tissues from the 17 canid species were divided into two samples that were analyzed separately. Species in the first sample represent a family-wide survey and include 11 species from 10 canid genera (Table 1). *Ursus arctos* (brown bear), the outgroup, is included as a twelfth species. Species in the second sample were intended to resolve relationships among more closely related taxa and include 11 species from two distinct groups: 1) the wolf-like canids, including five species and a South American canid as an outgroup; and 2) the *Vulpes*-like canids including five species (Table 1). Allozyme polymorphisms were scored and given letter designations separately in the family-wide and generic level surveys (Appendix 2). Alleles are not necessarily homologous between the two surveys and genetic distances were computed separately for samples 1 and 2.

We used the BIOSYS-1 program of Swofford and Selander (1981) to calculate Nei's (1978), Rogers' (1972) and Cavalli-Sforza and Edwards' (1967) chord distances. BIOSYS-1 was then used to generate separate UPGMA and distance-Wagner trees of species in samples 1 and 2 (Table 1). The topology of trees that were derived from these distance measures is similar. We present Nei's (1978) distance only. We chose a UPGMA tree based on Nei's genetic distance modified for small sample size and a distance-Wagner tree based on Cavalli-Sforza and Edwards' chord distance (Cavalli-Sforza and Edwards, 1967; Nei, 1978) because of simulations done by Nei et al. (1983). Their results suggest that 1) UPGMA and distance-Wagner trees generated with Cavalli-Sforza and Edwards' (1967) chord distance produce the most accurate branching patterns; and 2) Nei's distance (1978) gave the best estimate of branch lengths when used to generate a UPGMA tree. However, the Nei et al. (1983) results must be interpreted with caution because considerable controversy surrounds the use of distance data to estimate topologies and branch lengths (Farris, 1981, 1985, 1986; Felsenstein, 1984, 1986). The distance-Wagner tree was optimized to allow for negative branch lengths, which facilitates comparison of this tree to the UPGMA tree with the goodness-of-fit measures (Swofford, 1981; Hedges, 1986). Distance-Wagner trees for sample 1 and for the wolf-like canids were rooted using *Ur-*

TABLE 1. Group membership, scientific and common names, geographic range, source of tissues and tissue types of the canids analyzed in this study. Geographic range data from Nowak and Paradiso (1983). Source: CMZ, Catoctin Mountain Zoo, Frederick, Maryland; DZP, Denver Zoological Park, Denver, Colorado; JBZ, Johannesburg Zoo, Johannesburg, South Africa; NIHP, National Institute of Heath Animal Facility, Poolsville, Maryland; NZP, National Zoological Park, Washington, D.C.; PPZ, Potter Park Zoo, Lansing, Michigan; RDZ, Rio de Janeiro Zoo, Rio de Janeiro, Brazil; SAZ, San Antonio Zoological Garden, San Antonio, Texas; SDZ, San Diego Zoological Park, San Diego, California. Tissue: R = red blood cells, L = lymphocytes, C = transformed cultured cells.

Species (code)	Common name	Geographic range	Source	Tissue
Sample 1				
Ursus arctos (Uar)	Brown bear	Holarctica	NZP	R, L, C
Canis familiaris (Cfa)	Domestic dog	World wide	NIHP	R, L, C
C. lupus (Clu)	Grey wolf	Holarctic	SDZ	R, L, C
Speothos venaticus (Sve)	Bush dog	South America	NZP	R, L, C
Chrysocyon brachyurus (Cbr)	Maned wolf	South America	NZP	R, L, C
Dusicyon vetulus (Dve)	Hoary fox	South America	RDZ	R, L, C
Cerdocyon thous (Cth)	Crab-eating fox	South America	PPZ	R, L, C
Urocyon cinereoargenteus (Uci)	Grey fox	North America	CMZ	R, L, C
Octocyon megalotis (Ome)	Bat-eared fox	Africa	NZP	R, L, C
Vulpes vulpes (Vvu)	Red fox	Holarctic	CMZ	R, L, C
Fennecus zerda (Fze)	Fennec	North Africa	SAZ	R, L, C
Nyctereutes procyonoides (Npr)	Raccoon dog	Asia, Europe	DZP	R, L, C
Sample 2				
Wolf-like canids				
Canis familiaris (Cfa)	Domestic dog	World wide	NIHP	R, L, C
C. lupus (Clu)	Grey wolf	Holarctica	SDZ	R, L, C
C. latrans (Cla)	Coyote	North America	CMZ	R, L
Lycaon pictus (Lpi)	African wild dog	Africa	SDZ	R, L, C
C. mesomelas (Cme)	Black-backed jackal	Africa	JBZ	R, L
South American canid				
Chrysocyon brachyurus (Cbr)	Maned wolf	South America	NZP	R, L, C
Vulpes-like canids				
Fennecus zerda (Fze)	Fennec	North Africa	SAZ	R, L, C
Vulpes chama (Vch)	Cape fox	South Africa	JBZ	R, L
V. vulpes (Vvu)	Red fox	Holarctica	CMZ	R, L, C
Alopex lagopus (Ala)	Arctic fox	Holarctica	CMZ	R, L, C
V. macrotis (Vma)	Kit fox	Western U.S.	NZP	R, L, C

sus arctos and *Chrysocyon brachyurus*, respectively, as outgroups. The tree for the *Vulpes*-like canids was rooted at the midpoint. Two goodness-of-fit measures are presented here: Prager and Wilson's *F*-statistic (1976) and the cophenetic correlation coefficient (Sneath and Sokal, 1973; Nei, 1977). Finally, we used the CONTREE subroutine contained in the PAUP program by David L. Swofford (Version 2.4) to calculate the topology of a consensus tree from UPGMA and distance-Wagner trees of each group using the "strict" method outlined by Rohlf (1982).

RESULTS

Sample 1

Genetic distance values range from 1.387 (*Ursus arctos*/*Urocyon cinereoargenteus*) to 0.042 (*Canis lupus*/*C. familiaris*) (Table 2). Forty loci (78%) are polymorphic among the canids in sample 1. The outgroup *Ursus arctos* is genetically distant from all canids, the average distance is 1.16 ± 0.10 and the outlying values are 0.999 (*Chrysocyon*/*Ursus*) and 1.387 (*Urocyon*/*Ursus*). This suggests both an ancient divergence of this species from extant canids and, because of a relatively narrow range of values, a uniformity in the rate of protein evolution in the different canid lineages. The average distance value between canids is 0.33. The most closely related pairs are: *Canis familiaris* (domestic dog) and *Canis lupus* (grey wolf), 0.042; *Cerdocyon thous* (crab-eating fox) and *Dusicyon vetulus* (hoary fox), 0.101; and *Fennecus zerda* (fennec) and *Vulpes vulpes* (red fox), 0.131. Generally, the larg-

TABLE 2. Nei's distance (1978) (above diagonal) and number of loci examined (below diagonal) for species in group 1.

Species	*Ursus arctos*	*Canis familiaris*	*C. lupus*	*Speothos venaticus*	*Chrysocyon brachyurus*	*Dusicyon vetulus*	*Cerdocyon thous*	*Urocyon cinereo-argenteus*	*Otocyon megalotis*	*Nyctereutes procyo-noides*	*Vulpes vulpes*	*Fennecus zerda*
Ursus arctos (brown bear)	*****	1.166	1.177	1.179	0.999	1.089	1.144	1.387	1.172	1.194	1.078	1.143
Canis familiaris (domestic dog)	47	*****	0.042	0.215	0.267	0.231	0.341	0.299	0.406	0.439	0.432	0.390
C. lupus (grey wolf)	48	49	*****	0.240	0.292	0.208	0.292	0.240	0.412	0.434	0.426	0.385
Speothos venaticus (bush dog)	46	47	48	*****	0.235	0.334	0.292	0.342	0.420	0.470	0.409	0.398
Chrysocyon brachyurus (maned wolf)	48	47	48	46	*****	0.224	0.245	0.359	0.373	0.363	0.335	0.395
Dusicyon vetulus (hoary fox)	47	48	48	46	47	*****	0.101	0.242	0.360	0.446	0.337	0.388
Cerdocyon thous (crab-eating fox)	46	45	46	46	46	45	*****	0.285	0.338	0.465	0.401	0.486
Urocyon cinereoargenteus (grey fox)	47	47	48	46	46	47	45	*****	0.293	0.308	0.324	0.333
Otocyon megalotis (bat-eared fox)	45	46	46	44	46	47	43	45	*****	0.338	0.331	0.373
Nyctereutes procyonoides (raccoon dog)	45	45	46	44	46	44	43	44	43	*****	0.353	0.395
Vulpes vulpes (red fox)	48	49	50	48	48	48	46	48	46	46	*****	0.131
Fennecus zerda (fennec)	48	47	48	46	48	47	46	47	45	45	48	*****

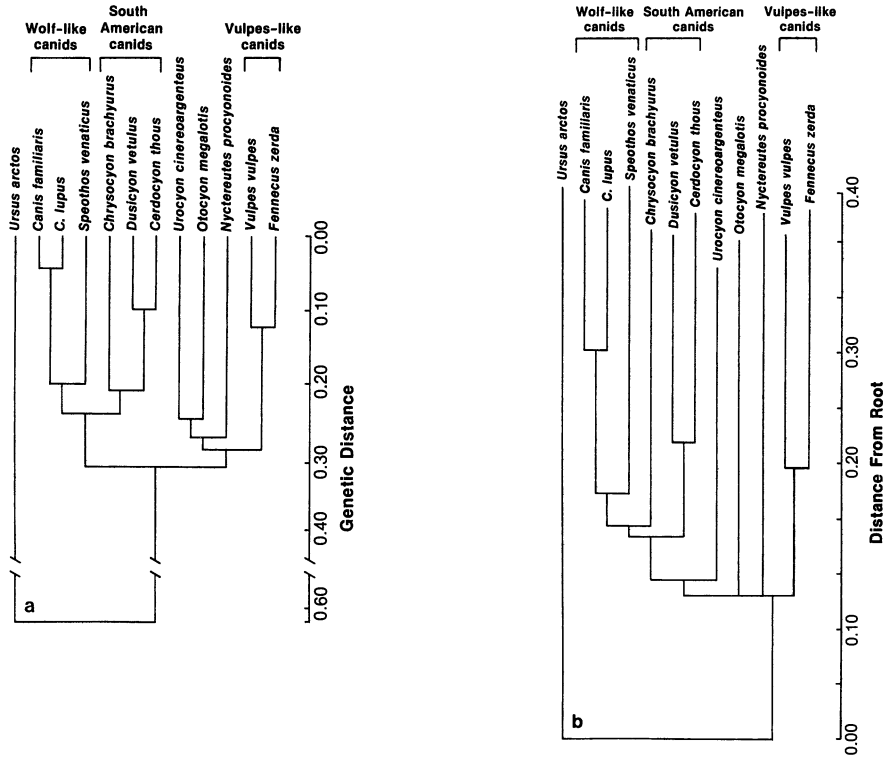

FIG. 1. a. UPGMA tree of the canids in group 1 based on Nei's distance (1978). Prager and Wilson's *F*-value = 7.1, the cophenetic correlation coefficient = 0.98. b. Distance-Wagner tree of the canids in group 1 based on Cavalli-Sforza and Edwards' (1967) chord distance. Prager and Wilson's *F*-value = 2.8, the cophenetic correlation coefficient = 0.99.

est distance occurred in comparisons of *Otocyon, Urocyon, Nyctereutes* and the other canids.

These distance values are reflected in the UPGMA phenogram and distance-Wagner tree (Fig. 1a, b), which exhibit broadly similar branching patterns. In both, *Canis familiaris* and *C. lupus, Cerdocyon thous* and *Dusicyon vetulus,* and *Vulpes vulpes* and *Fennecus zerda* are sister taxa. These species pairs are linked to other taxa so as to form several distinct groupings: the wolf-like canids, including *Canis familiaris, C. lupus* and at a low level of similarity, *Speothos venaticus;* the South American canids, including *Dusicyon vetulus* and *Cerdocyon thous* and, in the UPGMA tree, *Chrysocyon brachyurus;* and the *Vulpes*-like canids, including *Vulpes vulpes* and *Fennecus zerda.* In both trees, the wolf-like canids are most closely allied with the South American canids, together they possibly form a monophyletic grouping. The *Vulpes*-like canids are not closely associated with any other taxa in either tree. Similarly, species of the genera *Otocyon, Urocyon* and *Nyctereutes* are all genetically distinct and appear to have diverged early in the history of the family.

Differences between the UPGMA phenogram and the distance-Wagner tree concern the placement of the *Urocyon* lineage. The distance-Wagner tree suggests an unresolved tetrachotomy among *Nyctereutes, Otocyon,* the *Vulpes*-like canids and the group containing the wolf-like canids and the South American canids. The UPGMA tree agrees with the approximate three-way split of *Nyctereutes, Otocyon* and the *Vulpes*-like canids but does not associate *Urocyon* with the wolf-like canids or the South American canids. Because these nodes are

TABLE 3. Nei's genetic distance (1978) (above diagonal) and number of loci examined (below diagonal) for species in group 2.

Species	Wolf-like canids					
	Canis familiaris	*C. lupus*	*C. latrans*	*C. mesomelas*	*Lycaon pictus*	*Chrysocyon brachyurus*
Canis familiaris (domestic dog)	*****	0.013	0.050	0.176	0.131	0.203
C. lupus (grey wolf)	44	*****	0.036	0.193	0.131	0.261
C. latrans (coyote)	43	44	*****	0.240	0.084	0.264
C. mesomelas (black-backed jackal)	36	36	36	*****	0.356	0.409
Lycaon pictus (African wild dog)	39	39	39	34	*****	0.311
Chrysocyon brachyurus (maned wolf)	40	41	40	32	35	*****

Species	Vulpes-like canids				
	Fennecus zerda	*Alopex lagopus*	*Vulpes chama*	*V. macrotis*	*V. vulpes*
Fennecus zerda (fennec)	*****	0.170	0.180	0.105	0.102
Alopex lagopus (arctic fox)	39	*****	0.220	0.079	0.128
Vulpes chama (cape fox)	34	33	*****	0.206	0.169
V. macrotis (kit fox)	43	39	34	*****	0.145
V. vulpes (red fox)	44	40	34	44	*****

close and because it is difficult to compare goodness-of-fit measures from trees that optimize different criteria it is uncertain which tree is better.

Sample 2

Genetic distance among the wolf-like canids.—Several other species are commonly associated taxonomically with *Canis familiaris* and *C. lupus*, and these include *Canis latrans* (coyote), *Canis mesomelas* (black-backed jackal), and *Lycaon pictus* (African wild dog) (Clutton-Brock et al., 1976; Van Gelder, 1978; Nowak and Paradiso, 1983). Genetic distances among pairs of these wolf-like canids are generally small (Table 3). Overall, fewer loci were scored in this survey, which may have caused discrepancies in distance values between several taxa that are repeated in this survey (*Chrysocyon*/*Canis familiaris*, *Chrysocyon*/*C. lupus*, and *C. familiaris*/*C. lupus*; Tables 2, 3). Moreover, the number of informative loci among the wolf-like canids is fewer, only 13 (29%) of the loci are polymorphic.

The most similar taxa are *Canis familiaris* and *C. lupus* (0.013), which are both similar to *C. latrans* (0.036, *C. latrans*/*C. lupus*; 0.050, *C. latrans*/*C. familiaris*; Table 3). The remaining taxa generally show distance values greater than 0.100. Surprisingly, *Canis mesomelas* has relatively large genetic distance values between it and all the other wolf-like canids. As expected, the largest distance values are between *Chrysocyon brachyurus* and the other canid taxa.

Both the UPGMA phenogram and the distance-Wagner tree reflect these patterns of genetic similarity but differ from each other in the specifics of their branching order (Fig. 2). In both trees, *Canis mesomelas* diverged first followed by *Lycaon pictus* and *C. latrans* in the UPGMA tree. The distance-Wagner tree unites *Lycaon pictus* and *C. latrans* as sister taxa whereas the phenogram does not. *Canis familiaris* is closely linked with *C. lupus* in both trees.

Genetic distances among the Vulpes-like canids.—Distance values are less variable among the *Vulpes*-like canids (Table 3).

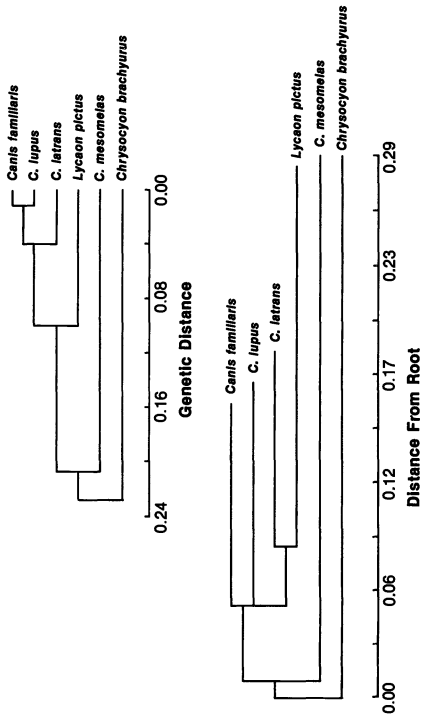

FIG. 2. UPGMA (left) and distance-Wagner (right) trees of the wolf-like canids in group 2 based, respectively, on Nei's distance (1978) and Cavalli-Sforza and Edwards' (1967) chord distance. The UPGMA and distance-Wagner trees of the wolf-like canids have, respectively, a Prager and Wilson's *F* of 20.0 and 4.6 and a cophenetic correlation coefficent of 0.88 and 0.98.

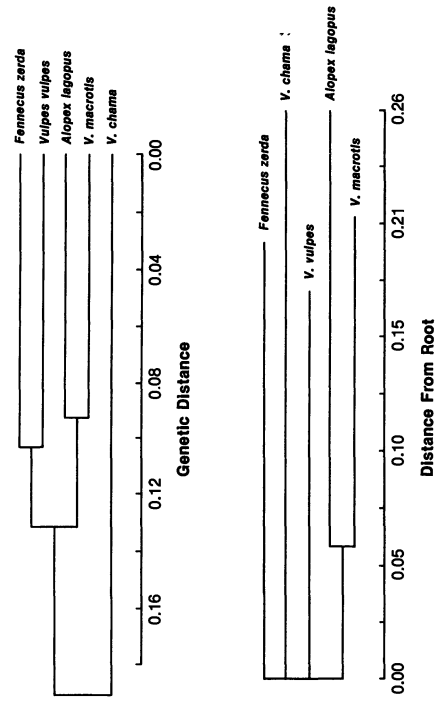

FIG. 3. UPGMA (left) and distance-Wagner (right) trees of the *Vulpes*-like canids in group 2 based, respectively, on Nei's distance (1978) and Cavalli-Sforza and Edwards' (1967) chord distance. The UPGMA and distance-Wagner trees of the *Vulpes*-like canids have, respectively, a Prager and Wilson's *F* of 10.6 and 2.8 and a cophenetic correlation coefficent of 0.90 and 0.95.

They range from 0.079 between *Vulpes macrotis* (kit fox) and *Alopex lagopus* (arctic fox) to 0.220 between *V. chama* (cape fox) and *A. lagopus* with a mean of 0.150. Seventeen (38%) of the loci are polymorphic among the *vulpes*-like canids. In both the UPGMA phenogram and distance-Wagner tree (Fig. 3), *A. lagopus* and *V. macrotis* are sister taxa. However, in the distance-Wagner tree the remaining taxa appear as an unresolved trichotomy radiating very close to the root of the tree. In the UPGMA phenogram, *Fennecus zerda* and *Vulpes vulpes* are placed in a group separate from that of *V. chama* and closest to a group containing *Alopex lagopus* and *Vulpes macrotis*.

Consensus Tree and Absolute Time

A time scale was added to a "strict" consensus tree (Rohlf, 1982) by assuming the branching point between the wolf-like canids and the South American canids occurred approximately 7 MYBP (Fig. 4). This time is based on the first appearance of *Canis davisii*, the potential ancestor of both groups, in the North American fossil record (Berta, 1984). Assuming that this is a reasonable divergence time for these two groups, 0.1 genetic distance (Nei, 1978) units equals approximately 2.5 million years, which is similar to values estimated for other vertebrate groups (Avise and Aquadro, 1981; Thorpe, 1982). However, considerable variability in the rate of protein evolution has been found among vertebrates (Avise and Aquadro, 1982; Thorpe, 1982; Kessler and Avise, 1985; Britten, 1986; Vawter and Brown, 1986). Because Canidae is a closely-related family it is hoped that the variability in the rate of protein evo-

lution among canid taxa is small. The constancy of protein evolution in the Canidae is suggested by the correspondence of divergence times and first appearance dates in the fossil record (see Discussion).

As with the previous family-wide trees three distinct lineages are suggested by the consensus tree: 1) the wolf-like canids including *Canis, Lycaon* and perhaps *Speothos;* 2) the South American canids, including *Dusicyon* and *Cerdocyon* and at a significantly greater level of divergence, *Chrysocyon;* and 3) the *Vulpes*-like canids, including *Vulpes, Fennecus* and *Alopex.* The remaining canid taxa (*Otocyon, Urocyon* and *Nyctereutes*) are not closely related to any of the canid species that were surveyed.

Among the wolf-like canids, differentiation began about 6 MYBP, and in our analysis is represented by an unresolved trichotomy among *Speothos venaticus, Canis mesomelas* and the remaining wolf-like canids (Fig. 4). The relative branching sequence of *C. mesomelas* and *Speothos venaticus* cannot be resolved because they were not included in the same survey (see Materials and Methods section). However, relative distance values suggest that *Speothos* is less closely allied to the *C. lupus–C. familiaris* group than is *C. mesomelas* (Tables 2, 3). A second branching event occurred approximately 3 MYBP and involved *Lycaon pictus, C. latrans* and the lineage leading to *C. familiaris* and *C. lupus.*

Among the South American canids *Chrysocyon brachyurus* is the earliest divergence at approximately 6.5 MYBP. The divergence of *Dusicyon vetulus* and *Cerdocyon thous,* approximately 2.5–3 MYBP, is roughly coincident with the opening of the Panamanian land bridge (Marshall et al., 1982, 1984). The genus *Dusicyon* includes five other species that were not available for analysis but are thought to be closely associated with *Dusicyon vetulus* (Langguth, 1969). The other endemic South American canid analyzed in this study, *Speothos venaticus,* appears not to be closely allied to the *Dusicyon* group.

Among the *Vulpes*-like canids only *V. macrotis* and *A. lagopus* are clustered together, their divergence time is approximately 2–3 MYBP. This cluster and the lineages leading to *V. chama, V. vulpes,* and *Fennecus zerda* diverged from each other approximately 5 MYBP.

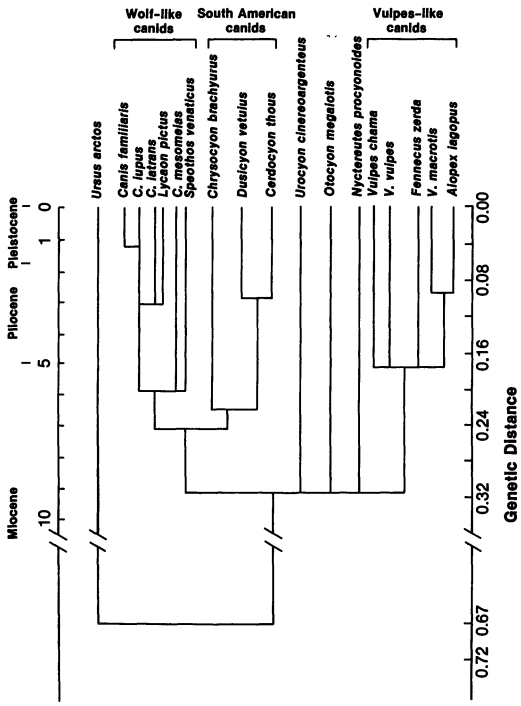

FIG. 4. A "strict" consensus tree (Rohlf, 1982) based on UPGMA and distance-Wagner trees of species in samples 1 and 2.

DISCUSSION

Evolution of the Trenchant Heel

The direction of trenchant heel evolution depends on whether the presence of a trenchant heel on the carnassial tooth is viewed as a primitive or derived character for the wolf-like canids. The latter appears more likely because the trenchant heel is not present in the potential ancestors of the wolf-like canids (Kurten, 1968, 1974; Kurten and Anderson, 1980). If this is the case, the trenchant heel has apparently evolved independently in *Speothos* and *Lycaon.* Alternatively, if this condition is primitive then it was secondarily lost in the other wolf-like canids. This degree of evolutionary flexibility suggests that the

trenchant heel condition may not be a highly conserved character.

Parallelism in both development of a trenchant heel and reduction of the post-carnassial molars may be the result of similar selective pressure for increased efficiency in processing meat (Ewer, 1973; Van Valkenburgh, in press). *Lycaon* and *Cuon* are large cursorial predators whose diet is predominately meat (Kingdon, 1977; Johnsingh, 1981). In both canids, trenchant heels are present on the carnassial and post-carnassial grinding molars are reduced, especially in *Cuon*. Surprisingly, the reduction of the post-carnassial molars is most extreme in the diminutive *Speothos*. The seemingly predacious dentition of *Speothos* is combined with a definitively non-cursorial skeleton: a long trunk with remarkably short, robust legs (Hildebrand, 1952, 1954; Langguth, 1969). This unusual combination of features has likely contributed to the confusion surrounding its diet and prey-killing behavior (Bates, 1944; Hildebrand, 1952; Langguth, 1969; Kitchener, 1971; Kleiman, 1972; Clutton-Brock et al., 1976; Brady, 1981; Deutsch, 1983). However, *Speothos* could be related to the extinct New World canid genus *Protocyon* which is much larger in body size (Berta, 1979; Kurten and Anderson, 1980). *Speothos* and *Protocyon* share several morphologic features including the presence of a trenchant heel. Thus, the apparent carnivorous dentition of *Speothos* could be only retained from a larger more carnivorous ancestor. Moreover, the phyletic decrease in body size that may have occurred in the evolution of *Speothos* could have resulted in other associated effects. Dwarfism has been observed in many mammal lineages to produce distinct allometric effects (cf. Marshall and Corruccini, 1978; Prothero and Sereno, 1982; Roth, 1984). For instance, small domestic dogs have relatively larger metatarsals than large dogs (Wayne, 1986b). Moreover, small animals may be mechanistically less able to accommodate morphologic features such as additional teeth or toes (Alberch, 1985). Thus, the unusual limb proportions and loss of post-carnassial teeth in *Speothos* might be a consequence of body size reduction or dwarfism rather than a specific dietary or locomotor adaptation.

The Radiation of the South American Canids

Our results suggest that the diverse array of morphologies represented by the recent South American canids has a complex origin. Apparently, the long-legged maned wolf *Chrysocyon* is not closely related genetically to any canid examined in this survey and thus appears to represent the sole, terminal species of a 6-million-year-old lineage. No fossil or living intermediates exist to connect this morphologically aberrant species with ancestral fossil forms. Similarly, *Speothos* is not closely associated genetically with other recent South American canids analyzed in this survey and appears more closely associated with the wolf-like canids. Apparently, the lineages leading to *Chrysocyon* and *Speothos* were genetically distinct well before the opening of the Panamanian land bridge and the radiation of the fox-like South American canids.

The radiation of the South American foxes (the *Dusicyon* group and *Cerdocyon thous*) began approximately 2–3 MYBP, an example of rapid morphologic evolution. This radiation coincided with the opening of the Panamanian land bridge in the Pliocene and may have been fostered by the absence of eutherian terrestrial predators in South America (Patterson and Pascual, 1972; Simpson, 1980; Marshall et al., 1982).

Relationship of These Results to Morphologic, Karyologic and Paleontologic Studies

A detailed discussion of morphologic, karyologic and paleontologic studies of the Canidae is given in Wayne et al. (in press). These studies support many aspects of the tree presented in Figure 4. Specific areas of agreement and disagreement are outlined below.

Morphologic studies.—In general, qualitative and quantitative morphologic studies of the Canidae support the groupings represented in the consensus tree (Huxley,

1880; Simpson, 1945; Lawrence and Bossert, 1967; Langguth, 1969; Clutton-Brock et al., 1976; Van Gelder, 1978; Nowak, 1979; Olsen, 1985; Wayne, 1986a, b). Specifically, the following elements are supported: 1) the close grouping of *C. familiaris* and *C. lupus*, and the clustering of these two taxa with *C. latrans*; 2) the close association of *Dusicyon vetulus* and *Cerdocyon thous* and their distant association with the other South American canids, *Chrysocyon brachyurus* and *Speothos venaticus*; 3) the association of species in *Vulpes* with *Fennecus zerda*; and 4) the distant association of *Urocyon, Nyctereutes*, and *Otocyon* to other canid taxa. The primary areas of disagreement concern: 1) the large genetic distance between *C. mesomelas* and the other wolf-like canids; 2) the association of *Speothos* with the wolf-like canids; and 3) close clustering of *V. macrotis* and *Alopex lagopus*. A cladistic tree of the South American canids presented by Berta (1984) differs principally from Figure 4 in that *Speothos* is shown as a sister taxon of *Nyctereutes procyonoides*. In her analysis, these taxa are part of a clade that includes *Cerdocyon* and *Dusicyon*.

Karyologic studies.—Standard and G-banded karyotypes have been described for many of the canids discussed in this study (Chiarelli, 1975; Wurster-Hill and Centerwall, 1982; Yoshida et al., 1983; Wayne et al., 1987a, b). Several groupings seen in Figure 4 are supported by the results of these studies: 1) the association of the wolf-like and South American canids, all of which have high diploid number karyotypes and a large number of small acrocentric chromosomes; 2) the association of *Canis lupus, C. familiaris, C. latrans* and *Lycaon pictus*, all of which share a derived diploid number of 78 and similar chromosome morphology; 3) the grouping of *Cerdocyon thous* with *Dusicyon vetulus* based on a common diploid number of 74 and extensive chromosome arm homology; 4) the close association of the *Vulpes*-like canids; except for *Fennecus*, all these canids have low-numbered karyotypes and a considerable degree of arm homology; 5) the close association between *V. macrotis* and *Alopex*, based on a unique, shared, G-band homologous karyotype; and 6) the distant association of *Urocyon, Nyctereutes* and *Otocyon* with other canids based on their unique karyotypes.

Paleontologic studies.—Both the time scale and branching order of Figure 4 are in large part supported by the fossil record. In agreement with Figure 4, the archaezoological record shows that *C. familiaris* is a very recent derivative of *C. lupus* (Scott, 1968; Epstein, 1971; Turnbull and Reed, 1974; Olsen, 1985). The common ancestor of these two taxa and *C. latrans* probably existed in the late Pliocene, 2 MYBP (Giles, 1960; Kurten, 1974; Nowak, 1979; Kurten and Anderson, 1980). The genus *Lycaon* first appears about 1.5 MYBP in Europe and Africa (Kurten, 1968; Savage and Russell 1983). The European species provides a potential link between the modern species and its presumed European *C. lupus*-like ancestors as suggested by Figure 4. The earliest record of *Dusicyon* and *Cerdocyon* in South America is approximately 1–2 MYBP, which is in near agreement with the consensus tree (Berta, 1979, 1984). The first appearance of *Speothos* and *Chrysocyon* is late Pleistocene, which is more recent than suggested by the consensus tree (Berta, 1984). However, their fossil record is poor and *Speothos* may be a descendant of *Protocyon* that was present in the late Pliocene of the New World (Berta, 1979; Kurten and Anderson, 1980). The first recognized *Vulpes* species in the fossil record is mid-Miocene (9–12 MYBP), which supports the divergence time of the *Vulpes*-like canids from the other canid species shown in Figure 4 (Savage and Russell, 1983). *Vulpes vulpes* and *V. chama* have fossil records extending back to the early Pleistocene, 1–1.8 MYBP, and the other *Vulpes*-like canids all appear more recently, 0.5 to 1 MYBP (Kurten, 1968; Kurten and Anderson, 1980; Savage and Russell, 1983). Figure 4 suggests earlier times of first appearance for these taxa. Finally, the fossil record of *Urocyon, Nyctereutes*, and *Otocyon* supports relatively early times of origination as suggested by Figure 4. *Nyctereutes* first appears 5 MYBP in the European fossil record, and *Urocyon* appears in the late Hemphillian,

4–6 MYBP (Kurten and Anderson, 1980; Savage and Russell, 1983; Berta, 1984). *Otocyon* has a sparse fossil record that extends as far back as the late Pliocene, 2 MYBP, of North Africa (Savage and Russell, 1983).

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APPENDIX 1. Gene-enzyme systems examined. Gene symbols are for homologous (where possible) symbols recommended by the nomenclature committee of the VIIIth International Workshop on Human Gene Mapping (McAlpine et al., 1985). The basis of homology with human systems is defined by Harris and Hopkinson (1976). Four buffer systems were employed and these include: 1) TEB: electrode, 0.18 M tris, 0.004 M EDTA, 0.1 M boric acid pH 8.6; gel 0.1× of electrode buffer; 2) TC: electrode 0.14 M tris, 0.043 M citric acid pH 7.1; gel 0.07× of electrode buffer; 3) TEM: electrode, 0.1 M tris, 0.01 M EDTA, 0.1 M maleic acid, 0.01 M $MgCl_2$ pH 7.4; gel 0.1× of electrode buffer; 4) TG: electrode 0.005 tris, 0.039 M glycine pH 8.9; gel 0.37 M tris HCl pH 8.9. Tissues used: R = red blood cells; L = lymphocytes; C = transformed cultured cells.

Enzyme	IUPAC-IUB No.	Gene symbol	Buffer system	Tissue used
1. Acid phosphatase-1	3.1.3.2	ACP1	TC	R, C
2. Acid phosphatase-2	3.1.3.2	ACP2	TC	R, L, C
3. Adenosine deaminase	3.5.4.4	ADA	TEB	L, C
4. Adenine phosphoribosyl transferase	2.4.2.7	APRT	TG	R
5. Adenylate kinase-1	2.7.4.3	AK1	TC	R, L, C
6. Aminoacylase-1		ACY1	TC	L, C
7. Carbonic anhydrase-1	4.2.1.1	CA1	TEB	R
8. Carbonic anhydrase-2	4.2.1.1	CA2	TEB	R
9. Catalase	1.11.1.6	CAT	TEB	R
10. Creatine kinase-B	2.7.3.2	CKBB	TEB	R, L, C
11. Diaphorase 1	1.6.4.3	DIA1	TEB	R, L, C
12. Diaphorase 4	1.6.4.3	DIA4	TEB	R, L, C
13. Esterase	3.1.1.1	ES1	TC	R, L, C
14. Esterase	3.1.1.1	ES2	TC	R, L, C
15. Glutamate oxaloacetate transaminase (soluble)	2.6.1.1	GOT1	TEB/TEM	L, C
16. Glutamate oxaloacetate transaminase (soluble)	2.6.1.1	GOT2	TEB/TEM	L, C
17. Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD	TEB	R, L, C
18. Glutamate pyruvate transaminase	2.6.1.2	GPT	TC	R, C
19. Glucose phosphate isomerase	5.3.1.9	GPI	TEB	R, L, C
20. Glutathione reductase	1.6.4.2	GSR	TEB	R, L, C
21. β-glucuronidase	3.2.1.31	GUSB	TC	R, L, C
22. Glyoxylase-1	4.4.1.5	GLO1	TEB	R, C
23. Hexosaminidase-A	3.2.1.30	HEXA	TEB	R, L, C
24. Hexokinase-1	2.7.1.1	HK1	TEB	R, L, C
25. Hexokinase-2	2.7.1.1	HK2	TEB	R, L, C
26. Isocitrate dehydrogenase-1 (soluble)	1.1.1.42	IDH1	TC	R, L, C
27. Isocitrate dehydrogenase-2 (mitochondrial)	1.1.1.42	IDH2	TC	R, L, C
28. Inosine triphosphatase	3.6.1.3	ITPA	TEB	R, L, C
29. Lactate dehydrogenase-A	1.1.1.27	LDHA	TC	R, L, C
30. Lactate dehydrogenase-B	1.1.1.27	LDHB	TC	R, L, C
31. Malate dehydrogenase-1 (soluble)	1.1.1.37	MDH1	TC	R, L, C
32. Malate dehydrogenase-2 (mitochondrial)	1.1.1.37	MDH2	TC	R, L, C
33. Malic enzyme-1 (soluble)	1.1.1.40	ME1	TC	R, L, C
34. Mannose phosphate isomerase	5.3.1.8	MPI	TEB	R, L, C
35. Nucleoside phosphorylase	2.4.2.1	NP	TC	R, L, C
36. Peptidase B	3.4.11.	PEPB	TC	R, L, C
37. Peptidase C	3.4.16.	PEPC	TC	R, L, C
38. Peptidase D	3.4.11.	PEPD	TC	R, L, C
39. Phosphyoglyceromutase-A	2.7.5.3	PGAM	TC	R, C
40. 6-Phosphogluconate dehydrogenase	1.1.1.43	PGD	TEB/TC	R, L, C
41. Phosphoglucomutase-1	2.7.5.1	PGM1	TC	R, L, C
42. Phosphoglucomutase-2	2.7.5.1	PGM2	TC	R, L, C
43. Phosphoglucomutase-3	2.7.5.1	PGM3	TC	R, L, C
44. Pyruvate kinase-1	2.7.1.40	PKM1	TEB	R, C
45. Pyruvate kinase-2	2.7.1.40	PKM2	TEB	R, C
46. Pyrophosphatase (inorganic)	3.6.1.1	PP	TEM	R, L, C
47. Superoxide dismutase-1	1.15.1.1	SOD1	TEB	R, L, C
48. Transferrin		TF	TG	serum
49. Triosephosphate isomerase	5.3.1.1	TPI	TEM	R, L, C
50. Albumin		ALB	TG	serum
51. Hemogloblin		HB	TEB	R

APPENDIX 2. Allozyme variation in the Canidae. Polymorphisms are scored separately in groups 1 and 2. Hence alleles may not be homologous between equivalent loci of the two groups. Dash indicates missing data. See Table 1 for definition of species codes.

	Group 1											
Gene symbol	Uar	Cfa	Clu	Cbr	Sve	Dve	Cth	Uci	Ome	Npr	Vvu	Fze
1. ACP1	B	A	A	A	A	A	A	A	C	A	A	A
2. ACP2	B	A	A/C	A	A	A	D	A	A	A	A	A
3. ADA	B	A	A	A	A	A	A	A	A	—	C	C
4. APRT	B	A	A	A	A	A	A	C	A	A	A	A
5. AK1	A	A	B	A	A	B	B	B	A	A	A	A
6. ACY1	—	A	A	—	C	A	—	A	A	—	B	—
7. CA1	A	A	A	A	A	A	A	A	A	A	A	A
8. CA2	B	A	A	A	A	A	A	A	A	B	A	A
9. CAT	A	A	A	A	A	A	A	B	A	A	A	A
10. CKBB	B	A	A	A	A	A	A	A	A	A	A	C
11. DIA1	A	A	A	—	A/B	A/C	C	A	—	—	A/C	A/D
12. DIA4	B	A	A	A	A	A	A	A	A	A	A	A
13. ES1	B	A	A	A	A	A/C	A	A	A/D	A	A	A
14. ES2	B	C	C	A	A	A	A	D	—	E	E	E
15. GOT1	B	A	A	C	A	C	A	A	A	A	C	—
16. GOT2	B	A	A	A	A	A	A	A	A	A	C	C
17. G6PD	A	A	A	A	B	A	A	A	A	A	A	B
18. GPT	B	A	A	A	A	—	A	A	—	A	C	C
19. GPI	B	A	A	C	A	C	C	C	C	C	C	C
20. GSR	B	A	A	A	A	A	A	A	A	C	A	A
21. GUSB	A	A	A	A	A	A	A	B	B	B	A	A
22. GLO1	B	A	A	B	A	A	A	A	A	A	A	A
23. HEXA	B	A	A	A	—	A	—	C	D	A	E	D
24. HK1	A	A	A	A	A	A	A	A	A	A	A	A
25. HK2	A	—	A	A	A	—	A	A	—	A	A	A
26. IDH1	B	A	A	A	A	A	A	A	A	A	A	A
27. IDH2	B	A	A	A	C	A	A	A	A	A	A	A
28. ITPA	B	—	B	A	A	B	—	A	A	A	A	A
29. LDHA	A	A	A	A	A	A	A	A	A	A	A	A
30. LDHB	B	B	B	B	B	A	A	A	A	A	A	A
31. MDH1	A	A	A	A	A	A	A	A	A	A	A	A
32. MDH2	B	A	A	A	A	A	A	A/C	A	—	A	A
33. ME1	B	A	A	A	C	D	D	E	F	E	E	E
34. MPI	A	B	B	A	A	A	A	A	A	A	A	A
35. NP	B	A	A	A	A	A	A	A	A	B	A	A
36. PEPB	B	A	A	C	C	A	C	A	A	A	A	A
37. PEPC	B	A	A	B	A	A	A	A	B	B	B	B
38. PEPD	B	C	A	B	D	B	B	A	A	A	A	A
39. PGAMA	A	A	A	A	A	A	A	A	A	A	A	A
40. PGD	A/B	A	A	A	A	A	A	A	C	A	A	A
41. PGM1	B	A	A	A	A	A/B	A	A	A/B	A	A	A
42. PGM2	—	A	A	B	A	A	C	A	C	B	A	A
43. PGM3	B	—	—	A	—	A	A	A	A	—	—	A
44. PKM1	A	A	A	A	A	A	A	A	A	A	A	A
45. PKM2	B	A	A	A	A	A	A	A	B	C	D	D
46. PP	B	A	A	A	A	A	A	A	A	C	A	A
47. SOD1	B	A	A	A	A	B/C	B	A	A	A	A	A
48. TF	B	A	A	C	A	D	D	—	E	F	G	H
49. TPI	A	A	A	A	A	A	A	A	A	A	A	A
50. ALB	B	A	A	A	A	A	A	—	A	A	C	C
51. HB	B	A	A	A	—	A	—	A	C	A	C	A

APPENDIX 2. Continued.

Group 2										
Cfa	Clu	Cla	Cme	Lpi	Cbr	Fze	Ala	Vch	Vma	Vvu
A	A	A	A	A	A	A	A	A	A	A
A	B	C	A	C	A	A	A	A	A	A
A	A	A	—	A	A	B	B	B	B	B
A	A	A	A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	C	A	A	A
—	—	—	—	—	—	—	—	—	—	—
A	A	A	A	A/B	A	A	A	A	A	A
A	A	A	A	A	A	A	A	A	A	A
—	—	—	—	—	—	—	—	—	—	—
A	A	A	A	A	A	A	A	A	A	A
A	A	A	A	A	—	A/C	A	A/C	C	A
A	A	A	—	A	A	A	A	—	A	A
A	A	A	A	A	A	A	A	A	A	A
A	A	A	A	A	B	C	A/D	C	A/D	C
—	—	—	—	—	—	—	—	—	—	—
A	A	A	A	—	A	B	B	—	B	B
A/B	A	A	A	A	C	C	C	C	C	A
A	A	A	A	A	—	A/B	A	—	—	B
A	A	B	A	B	B/C	B	B	B	A/B	B
A	A	A	A	A	C	A	A	A	A	A
A	A	A	A	A	—	—	A	—	B	A
A	A	A	A	A	B	A	A	A	A	A
A	A	A	—	A	A	B	—	C	C	C
A	A	A	B	A	A	A	A	—	A	A
—	—	—	—	—	—	—	—	—	—	—
A	A	A	—	—	A	—	A	—	A	A
A	A	A	A	A	A	A	A	B	A	A
A	A	—	—	—	A	A	B	—	B	A
A	A	A	A	A	A	A	A	A	A	A
A	A	A	A	A	A	B	B	B	B	B
A	A	A	B	A	A	A	A/B	A/B	A	A/B
A	A	A	—	A	A	B	—	—	B	B
A	A	A	—	A	A	B	—	—	B	B
A/B	A/B	B	—	—	C	C	B/D	D	B	C/D
A	A	A	A	B	A	A	A	A	A	A
A	A	A	A	A	B	A	A	C	A	A
A	A	A	B	A	C	A	A	A/C	A	A
A	A	A	A	A	—	A	B	A	A/B	A/B
A	A	A	A	A	A	A	A	A	A	A
A	A	A	A	A	A	A	A	A	A	A
A	A/B	A/B	C	A	A	A	A	A	A	A
—	—	—	—	—	—	—	—	—	—	—
—	A	A	—	—	B	B	—	—	B	B
A	A	A	A	—	A	A	—	—	A	A
A	A	A	A	B	A	B	B	B	B	B
A	A	A	B	A	A	A	A	C	A	A
A	A	A	A	A	A	A	A	A	A	A
A	A	A	B	C	D	E	D/F	G	F	H
A	A	A	A	A	A	A	A	A	A	A
—	—	—	—	—	—	—	—	—	—	—
A	A	A	A	A	A	A	A	A	A	A