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The thirty-eight extant species of the family Felidae have been placed in as few as two and as many as nineteen separate genera. The purpose of the present study, therefore, is to review the various classification schemes within the Felidae and to examine their validity on the basis of a different set of taxonomic characters than has been used before in determining kinship among the cats.

Because protein molecules are primary gene end-products, that is their amino acid sequences are the direct translation of the DNA-mRNA nucleotide sequences, it seems logical to assume that comparisons among homologous proteins from different organisms should provide useful systematic data. Therefore, this study has employed comparisons of the electrophoretic patterns of specific homologous protein components of the blood of eighteen felid species. The various proteins have been separated by mobility and staining techniques. The comparisons of the relative mobilities of these homologous components have indicated the degree of similarity among the eighteen species in terms of the blood proteins examined.

The species tested include twelve representatives of the smaller cats, two lynxes, three great cats and the cheetah. Separations have been run for hemoglobin, the hemoglobin-haptoglobin complex, albumin, esterases, lactic dehydrogenase, malic dehydrogenase, alkaline phosphatase, ceruloplasmin and transferrins. Initial analysis of the data collected has been made using multivariate discriminant analysis.

The analysis has indicated that the existing Felidae might be validly allocated to three genera: Felis, Panthera and Acinonyx. However samples of several systematically controversial species have not been tested. Therefore, no final conclusions can be made as to whether all thirty-eight extant species can be allied in those three genera until the blood proteins of these cats have been examined.

GENERIC RELATIONSHIPS OF EXTANT CATS: AN ELECTROPHORETIC STUDY OF BLOOD PROTEINS

by

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INTRODUCTION

A review of existing literature on felid systematics shows a well-defined family of thirty-seven wild species and the domestic house cat, Felis catus. The family has almost worldwide distribution, being found everywhere except Antarctica, Australia, New Zealand, Madagascar and several other small South Pacific islands and the West Indies. Although almost all types of environments have been found to be able to support members of the family, the most typical feline habitat seems to be one of woodlands or rough terrain rather than one of open terrain.

Despite their widespread distribution and diverse choice of habitat, the cats share many common morphological, physiological and behavioral characteristics; for it seems that the general cat type, once established, has undergone relatively little structural modification. All felids are carnivorous, vegetable matter being only a small part of their diet. Prey is usually captured by stalking or lying in wait and then overtaking the victim with a sudden burst of speed. All are digitigrade and have five digits on the fore foot and four on the hind foot. In all species except Acinonyx jubatus, the cheetah, the claws are retractile, sharply curved and sheathed between lobes of skin. In A. jubatus the claws are only semi-retractile, although they are thought to be fully retractile in the young of that species. The felid braincase is relatively large making the head somewhat rounded. The jaws extend into only a short snout, vision seeming to play a more important role in their survival as predators than smell. The felid dental formula is

$\frac{3,1,2-3,1}{3,1,\ 2,\ 1}$. The tongue is covered with horny papillae used to groom the animal's fur and help retain food within its mouth. The bursa of the ears are well-developed.

These characters that make the felids such an easily definable family have caused a great deal of controversy concerning their classification at the generic level (Table 1). In general, the controversy seems to be between the lumpers and the splitters; those who choose to lump the species into as few as two genera and those who split them up into as many as nineteen or more. In addition there is considerable disagreement about felid nomenclature. The only generic name which seems to have universal acceptance is Felis Linnaeus, 1758. However, there is a great deal of variation among authors as to the species to be included in that genus. On the other hand, the cheetah is consistantly assigned to a genus separable from all the other cats, but the name given to that genus is variable. Other than in specific citation of another author's synonomies, all references to species herein will be as per Walker et al. (1964).

Review of Felid Systematics

Gray in 1821 began a series of publications dealing with the collection of Mammalia held in the British Museum of Natural History in which he proposed a number of generic and subgeneric names for the felids in that collection. In 1867 he published a rather complete listing of the cats that he had catalogued. Using basically the general physical appearance, slight variation in skull formation and the shape of the pupil of the eye as characters, Gray determined that extant cats should

TABLE 1. Comparison of various approaches to generic classification of Felids

Mivart (1882)	Simpson (1945)	Anderson & Jones (1957)	Walker (1954)	Gray (1867)	Pocock (1917, 1951)	Ewer (1973)
				Chaus	Felis	Felis
					Leptailurus	Leptailurus
					Otocolobus	Otocolobus
				Viverriceps*	Prionailurus	Prionailurus
				Pardalina	Zibethailurus	
						Mayailurus
				Felis**	Leopardus	Leopardus
			Felis		Herpailurus	Herpailurus
						Lynchailurus
				Pajeros	Dendrailurus	Oncifelis
						Oreailurus
	Felis	Felis		Viverriceps*	Ictailurus	Ictailurus
				Catolynx	Pardofelis	Pardofelis
				Leopardus*	Profelis	Profelis
Felis					Puma	Puma
			Lynx	Lyncus	Lynx	Lynx
				Caracal		Caracal
		Neofelis	Neofelis	Neofelis	Neofelis	Neofelis
	Panthera			Leopardus*		
			Panthera	Tigris	Panthera	
		Leo		Leo		Panthera
			Uncia	Uncia	Uncia	
Cynailurus	Acinonyx	Acinonyx	Acinonyx	Gueparda	Acinonyx	Acinonyx

* Species which Gray included in the genera Viverriceps and Leopardus were considered by Pocock and Ewer to be not closely enough related to be allied in the same genera. Thus the duplication of these genera in Gray's classification indicates how the two have been subdivided by Pocock and Ewer.

** Although most of the species included in the first eight genera listed in Pocock's classification above were included in Gray's Felis, some species from four of these genera were also placed by Gray in the genera Chaus, Viverriceps, Pardalina and Pajeros as indicated.

be represented by fourteen genera. His effort resulted not in clarifying felid systematics but in adding more confusion to the nomenclature and in allying many unrelated species and generically separating many that seemed closely related. For example, the cougar was included in the same genus (Leopardus Gray, 1842) as the leopard and the jaguar. The lion (Leo Oken, 1816), tiger (Tigris Oken, 1816), snow leopard (Uncia Gray, 1867) and clouded leopard (Neofelis Gray, 1867) were each listed as separate genera. Moreover, a number of individuals that appear from their descriptions to be color morphs or geographic subspecies of the same species were often identified as different species in separate genera. Gray did, of course, place the cheetah in a separate genus which he named Guepardа Gray, 1867. (Gray 1837, 1842, 1867a, 1867b, 1867c).

In contrast with Gray's work, Mivart (1882) in organizing the suborder of carnivores, Aeluroidea (cats, viverrids and hyenas) concluded that only the cheetah was distinctive enough from the rest of the cats to be given separate generic rank (Cynailurus Wagler, 1830). Mivart distinguished the cheetah from Felis because of its semi-retractile claws and the rudimentary condition of the internal cusp of P^4. Elliot (1883) who dealt only with the cats concurred with Mivart, as did Lydekker (1894) in his Hand-book to the Carnivora.

Pocock (1917), despite Mivart's example, proved to be an even more extreme splitter than Gray (1867a), determining that there should be seventeen genera of felids. His work, however, seemed much more credulous than Gray's, and he was critical of those who seemingly ignored what must have appeared to him obvious relationships.

> "...my main purpose in publishing what follows [a classification of existing Felidae] has been to show the true relationship of the species to one another, so far as it can be determined, and to dispose of such prevalent but fictitious groupings as those which imply that the lion (leo) and the puma (concolor) are closely allied and that the lynx (lynx) differs more from the domestic cat (catus) than the latter differs from the tiger (tigris)." [op cit.]

Pocock appeared to base his classification mainly on four characters; the structure of the hyoid apparatus, the tympanic bulla, the feet and the rhinarium. The imperfect ossification of the hyoid apparatus in the lion, tiger, jaguar, leopard and snow leopard, he felt, distinguished them from the rest of the cats. He placed these species into two genera; the latter in the genus Uncia and the remaining in Panthera Oken, 1816. Pocock viewed the absence of cutaneous sheaths into which the claws could be drawn as indication that the cheetah should be placed as the single species in the genus Acinonyx Brookes, 1828. The remaining species, he determined, were so variable in the aforementioned characters that they should be divided into fourteen genera (Pocock, 1916a, 1916b, 1916c).

In 1951 the first volume of what was to be Pocock's complete monograph of the Felidae was published. Due to his death in 1947, the work was not completed. The first volume, however, dealt with the genera he designated as Felis and Otocolobus Brandt, 1842. Only slight revisions were made in those two genera from his 1917 work. He grouped four of his 1917 species, ocreata, ornata, caudata and shawiana together as subspecies of a single species, F. lybica (sic.), the African wild cat. He elevated F. bieti and F. margarita to specific rank from subspecies under F. chaus and F. ocreata, respectively. Finally he changed the name of the genus Trichaelurus Satunin, 1905 to Otocolobus, an earlier synonym.

J.A. Allen (1919) in surveying the systematic status of the "smaller spotted cats of tropical America" may have been the greatest splitter of all. He divided what he determined were twelve (but now appear to be only seven) species into seven separate genera. He was very critical of lumpers, feeling that they minimized the differences between the various species of cats. He expressed his concept of the purpose of generic ranking in the following statement. "A future use of generic divisions would not obscure the fact that they are all cats but would indicate that at least all cats are not alike, and perhaps inspire an interest as to how they differ." (op cit.). Although this statement would explain Allen's extreme division of the family, it seems to differ substantially from the generally accepted concept of generic classification.

In another partial classification of the felids, G.M. Allen (1939) attempted to order the African cats. He also drew criticism from Pocock (1951) "on the failure to express with any approach to accuracy the kinship between many of the genera of Felidae". He mistakenly placed the Fossa (Cryptoprocta Bennett, 1833), a viverrid, in the family. He also admitted three additional genera; Acinonyx, Caracal Gray, 1843 and Felis. This classification clearly implied that the lion and leopard were more closely related to the African wild cat than was the caracal, the African lynx.

Using a similar approach, Hall and Kelson (1959), who dealt only with the classification of North American Mammalia, placed the eight cat species in that group in only two genera, Felis and Lynx Kerr, 1792. Here

again the jaguar was considered more closely related to the ocelots, the cougar and the jaguarondi than were the bobcat and the Canadian lynx.

Simpson (1945) admitted three genera of the Felidae; Felis, Panthera and Acinonyx. He didn't list the species included in these genera but indicated through descriptions of synonomous genera, ranges, etc. what groups were to be included in each. Felis included the domestic cat, all species of smaller wild cats, the cougar and the lynxes. To Panthera he assigned lions, tigers, leopards, jaguars, the snow leopard and the clouded leopard. Again only the cheetah was placed in Acinonyx.

Anderson and Jones (1967) also indicated only generic not specific relationships of the felids in surveying the distribution and classification of recent mammals. They recognized four genera of cats; Felis, Leo, Brehm, 1829 Neofelis and Acinonyx. They allied the smaller cats and the lynxes as did Simpson (1945). However, they determined that the great cats could be divided into two very closely related genera; Leo* Brehm, 1829 (= Panthera, invalid) and Neofelis. They included in Leo all of the great cats except the clouded leopard (Neofelis).

Walker et al. (1964), however, again separated the lynxes (Lynx) from the other smaller cats (Felis). They also considered the clouded leopard (Neofelis) and the snow leopard (Uncia) to be separable from Panthera. As with the other authors surveyed, they placed the cheetah (Acinonyx) in a separate genus.

Following what appeared to be a trend toward lumping species into fewer genera, a recent work by Ewer (1973) has reverted to a very Pocock-

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*not synonomous with Gray's (1867) Leo Oken, 1816 which included only the lion.

like scheme. She made only a few modifications in Pocock's (1917, 1951) classification. She subdivided his Lynx into two genera, Lynx and Caracal and grouped his Prionailurus Severtzow, 1858 and Zibethailurus Severtzow, 1858 into a single genus, Prionailurus, and Uncia and Panthera into Panthera. In addition, she admitted two species not in Pocock's scheme and gave each separate generic rank, Mayailurus* Imaizumi, 1969 and Oreailurus Cornalia, 1865. The one major difference was in her classification of the small tropical American cats (Table 1).

Validity of Electrophoretic Approach to Systematics

Obviously there is much confusion over felid systematics. The classical approach to classification has been that of comparative morphology. Since, by definition, closely related species have genetic codes that are similar in many respects, the phenotypic expression of the genetic code should be very similar. Therefore, the assumption has been made that the more morphological characters two species have in common, the more closely related they are. As systematists have turned to other disciplines (comparative physiology, biochemistry, serology, behavior, etc.) for clues to relatedness, the assumption has continued to be applied that the more similar two species are in any measurable characters, the more closely related they are. Following this premise, then, it would seem that as the amino acid sequences of protein molecules are the direct translation of the nucleotide sequences of the genetic material, comparisons of homologous proteins from different organisms should be even more indicative of genetic relatedness than comparisons of such features as gross

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*Mayailurus iriomotensis, a new species described by Imaizumi in 1969, could be a geographic subspecies of the leopard cat (F. bengalensis).

morphological characters (Sibley, 1960, 1962, 1964, 1965, 1967; Zuckerkandl and Pauling, 1965; Johnson 1968 and Johnson and Wickes, 1959, 1964). Therefore, it is hoped that electrophoretic investigations of several blood proteins may give some indication of the genetic relatedness of the felids and thereby may help settle some of the controversy concerning their generic rank.

MATERIALS AND METHODS

The electrophoretic patterns of seventy blood specimens representing sixty different individuals were examined. These specimens constituted seventeen of the thirty-seven species generally accepted as making up the family Felidae plus F. catus (Walker et al., 1964; Ewer, 1973). Table 2 lists those species examined electrophoretically.

TABLE 2. Species examined electrophoretically

Species	Individuals Examined	Sources of Blood Proteins
Felis catus	9	Hemolysate, plasma
F. libyca	1	Serum
F. chaus	8	Serum
F. serval	4	Serum
F. bengalensis	2	Serum
F. viverrina	6	Serum
F. temmincki	4	Serum
F. concolor	4	Serum
F. pardalis	1	Hemolysate, plasma
F. wiedii	1	Hemolysate, plasma
F. colocolo	2	Serum
F. geoffroyi	1	Serum
Lynx rufus	1	Hemolysate, plasma
L. caracal	5	Serum
Panthera leo	2	Serum
P. tigris	5	Serum
P. pardus	2	Serum
Acinonyx jubatus	1	Serum

Samples of whole blood were collected from wild species of cats held at the Circle M Zoo, Stuart, Virginia and from a number of domestic cats. They were collected in plastic hypodermic syringes from the brachial vein using heparin as an anticoagulant. The plasma was separated from the red blood cells by centrifugation and after washing four to six times in 1% saline solution, the red blood cells were lysed by the addition of approximately 3 volumes of distilled water. Serum samples were obtained from reserves kept at the U.S. National Zoological Park, Washington, D.C. Plasma, hemolysate and serum were frozen immediately after preparation and retained in that condition.

Electrophoretic comparisons were carried out on vertical polyacrylamide gel equipment (E-C Technical Bulletin, 128). Various buffer systems and staining techniques were used in an attempt to separate and identify such substances as hemoglobin (Hb), hemoglobin-haptoglobin complexes (Hb-Hp), albumin, esterase, ceruloplasmin, transferrins, alkaline phosphatase, lactic dehydrogenase (LDH) and malic dehydrogenase (MDH), all of which have been reported to occur at various levels in the blood of some mammals and therefore could conceivably be found in felid blood.

Electrophoretic Separation and Staining Techniques

Hemoglobin was examined using a continuous buffer system after Peacock et al. (1965). A 7% gel was used instead of the 5% gel as described by Peacock. Samples were run anodally for four hours at 300 volts (80-120 ma.). A discontinuous system as described in E-C Technical Bulletin 141 was also used to separate hemoglobin. Amido

black 10B, a general protein stain, was used with both types of preparations. In addition, a second series of gels was prepared by the discontinuous system and was stained with a peroxidase stain, a hemoglobin-specific stain (op cit.).

Haptoglobins were examined using the same discontinuous system as that outlined for hemoglobin separation. As it has been determined that haptoglobin will complex with hemoglobin more strongly than any plasma (or serum) protein, it can be assumed that any hemoglobin (at least 1-3 mg. per 100 ml. can generally be found in most mammalian plasma, Laurell, 1960) present in plasma or serum will be complexed with haptoglobin. Therefore, the hemoglobin-haptoglobin complex may be detected in plasma or serum by the use of the hemoglobin-specific peroxidase stain.

The separation of albumin was accomplished through the use of a discontinuous buffer system. The gel buffer used was Tris-EDTA-Borate pH 8.4; the electrode chamber buffer, Tris-EDTA-Borate pH 9.6 (E-C Technical Bulletin 134). The samples were run anodally for three hours at 300 volts (80-120 ma.). The gel slab was stained with Amido black 10B which gave a total protein pattern. However, albumin, being the fastest of the plasma proteins, was easily distinguishable from the other protein bands.

Esterase separations were made as per Shaw and Koen (1968) and were run anodally for two and one-half hours at 300 volts (80-110 ma.). The enzyme separation was stained using as a substrate 1-naphthyl acetate and substituting Fast blue stain B for Fast blue RR (op cit.).

The copper-carrying ceruloplasmin was demonstrated by an O-diansidine staining technique after separation in an acetate buffer system pH 5.7

(Jensen, 1963 as reported in Wieme, 1965). A 5% gel was used and was run anodally for four hours at 200 volts (240-360 ma.).

Two systems were used to separate and stain transferrins. A dinitroso-naphthalenediol stain (Ornstein, 1966) was used with 5% gels run anodally in an acetate buffer system pH 5.7 at 200 volts (280-320 ma.) for four hours and with 7% gels run anodally in a tris-glycine buffer system pH 9.3 at 200 volts for three hours. In addition, both 5% and 7% gels, run anodally in a discontinuous buffer system (Poulik, 1957) for three and one-half hours at 200 volts, were stained using Nitroso R salt solution (Muellar et al., 1962).

Alkaline phosphatase detection was attempted using the techniques described in E-C Technical Bulletin 146. An α-naphthyl acid phosphate procedure (Taswell and Jeffers, 1963) was used for staining.

Separation and staining of LDH was carried out as per directions in E-C Technical Bulletin 144. MDH separations were carried out using the same electrophoretic conditions as for LDH. The same staining technique was used by substituting for sodium lactate sodium-L-malate as the substrate (Shaw and Koen, 1968).

Method of Analysis

The determination of similarities and differences between the different cat species was made by measuring the distance each component had migrated from the origin. In order to make allowances for slight differences in concentration of the gels, running times, and other such variables, which have been known to affect the absolute mobilities of the components, a standard was run in each gel along with the cat material.

The standard used in all gels was human material collected from a single individual. Reference (R_f) values were then computed for the mobility of each measured band of the felid specimens relative to the mobility of the standard in the same gel. Whenever possible (i.e. for haptoglobin, albumin, esterase and ceruloplasmin) the albumin leading edge of human plasma or serum was used as the standard. In the case of hemoglobin and LDH, where no albumin bands were present, the leading edge of the fastest hemoglobin or LDH band of the human material was used as the standard.

The resulting data was evaluated using multivariate discriminant analysis (Dixon, 1971). The packaged computer program used permitted analysis of the validity of certain classification schemes on the basis of as many as twenty-five variables. The input required by the program consisted of the set of test samples for each group specified (individuals of the cat species assigned to a proposed genus) along with the values for the set of variables used to classify the samples (the relative mobilities of the various blood proteins examined for each individual cat).

The program directed the computation of a set of linear functions for each group established by the investigator from the variables recorded for all samples examined. The model from which the functions were derived was a multivariate normal distribution of samples within groups such that the covariance matrices were the same for all groups.* The formula used to compute the set of functions for each group i follows.

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*The computer output from this program includes a chi-square value which can be used to test the hypothesis that the mean values of the matrices are the same for all groups.

$$f_i = \sum_{l=1}^{p} x_p c_{1i} + c_{0i} \qquad \text{Equation 1*}$$

where x_p = values of the variables for each sample,

c_{1i} = functional coefficient of each variable for the group i,

c_{0i} = functional constant for the group i.

Those variables having high intergroup consistency or having low intragroup consistency were weighted less heavily, i.e. were given a smaller functional coefficient, than those having low intergroup consistency. Thus the differences between groups were emphasized. However a functional constant was also computed for each group in order to provide for the equilization of the covariance matrices for all groups.

Given the set of functions for each group i (proposed genus), the probability of a given test sample (individual cat) being in group i was then determined using the following equation.

$$P_j = \frac{e^{(f_i - \max f_i)}}{\Sigma e^{(f_i - \max f_i)}} \qquad \text{Equation 2*}$$

where j = test samples 1, 2,....n.

Each sample was then assigned to the group for which its estimated probability density (P_j) was the largest (op cit.).

Each sample was placed in a suitable subspace (genus) of the space occupied by all the samples. Therefore, the probable generic relationships

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*Having these equations also gives a certain predictive value to the analysis, in that new data can be plugged into the equation for a particular set of groups. Then the group in which the new individual has the largest probability of occurring can be determined, although not quite as accurately as for data run through the program.

between the various individuals was based on the analysis of this n-dimensional subspace (where n= number of blood protein mobilities examined for the individual). Group assignments were thus made by determining the subspace in which the individual sample had the largest probability of being found. (Cooley and Lohnes, 1971)

Five different methods of grouping the eighteen species whose electrophoretic blood protein mobilities had been examined were subsequently analyzed. (Table 3) Although only R_f values for the various blood protein mobilities were used as variables to compute the linear functions, other taxonomic characters were used to set up the initial groups. To that end, any of the previously described systematic schemes for grouping the felid species into genera which appeared to have been based on valid interpretations of a variety of taxonomic characters (Mivart, 1882; Simpson, 1945; Walker et al., 1964; Anderson and Jones, 1967) were subjected to analysis, with the exception of certain extreme cases of splitting (Pocock, 1917, 1951; Ewer, 1973). Although these excepted schemes probably do represent relatively valid attempts at grouping closely related species, the resulting groups seemed too greatly subdivided to be valid genera.

The first trial grouping was based on the classification by Walker et al. (1964), the second on Simpson's (1945) classification scheme and the third on that of Mivart (1882). The fourth and fifth groupings were designated to test the hypothesis that the smaller cats of the Old World evolved separately from those of the New World and thus should be admitted to different genera. Trial four assumed that the lynxes are separable from the smaller cats. Trial five, on the other hand, assumed

TABLE 3. Possible generic groups to be tested by multivariate discriminant analysis

Trial I

Group 1	Group 2	Group 3	Group 4
Felis catus	Lynx rufus	Panthera pardus	Acinonyx jubatus
F. concolor	L. caracal	P. tigris	
F. libyca		P. leo	
F. chaus			
F. serval			
F. viverrina			
F. bengalensis			
F. temmincki			
F. pardalis			
F. wiedii			
F. colocolo			
F. geoffroyi			

Trial II

Group 1	Group 2	Group 3
Felis spp.	Panthera spp.	Acinonyx sp.
Lynx spp.		

Trial III

Group 1	Group 2
Felis spp.	Acinonyx sp.
Lynx spp.	
Panthera spp.	

Trial IV

Group 1	Group 2	Group 3	Group 4	Group 5
F. catus	F. concolor	Lynx spp.	Panthera spp.	Acinonyx sp.
F. libyca	F. pardalis			
F. chaus	F. wiedii			
F. serval	F. colocolo			
F. viverrina	F. geoffroyi			
F. bengalensis				
F. temmincki				

TABLE 3 Continued

Trial V

Group 1	Group 2	Group 3	Group 4
F. catus	F. concolor	Panthera spp.	Acinonyx sp.
F. libyca	F. pardalis		
F. chaus	F. wiedii		
F. serval	F. colocolo		
F. viverrina	F. geoffroyi		
F. bengalensis	L. rufus		
F. temmincki			
L. caracal			

that the lynxes are closely enough related to the other small cats to require inclusion in the same genera. That is, old world lynxes would have evolved separately from the new world lynxes but along with the geographically respective small cat species.

RESULTS

Electrophoretic Patterns

The discontinuous system proved to be the more satisfactory of the two systems used for hemoglobin separation. The separation resulted in two relatively dense protein bands both of which stained as hemoglobin using the Hb-specific peroxidase staining technique (E-C Technical Bulletin 141). Although hemolysate could be obtained for only four species, F. catus, F. pardalis, F. wiedii and L. rufus, the mobility patterns of these four varied little, the faster component ranging from 102 to 104 R_f units and the slower from 91 to 95 R_f units. However, since a large number of the species being examined did not have hemoglobin patterns available, hemoglobin separations were not used as a taxonomic character to determine relatedness in this study (Figure 1).

The hemoglobin-haptoglobin complex separated into two distinct bands. There was a good deal of variability in mobilities of different groups. In the Felis and Lynx species the faster component ranged from 44 to 79 R_f units and the slower from 39 to 72 R_f units. The components seemed slightly slower in the Panthera species, varying from 42 to 47 R_f units for the faster component and 36 to 42 for the slower. The single representative of the species A. jubatus showed a migrational pattern of 63 R_f units for the faster component and 47 for the slower (Figure 2).

The albumin preparation was detected as a single broad but rather diffuse band. There was no evidence of a pre-albumin band in patterns of any of the species. A good deal of variability was noticed within the

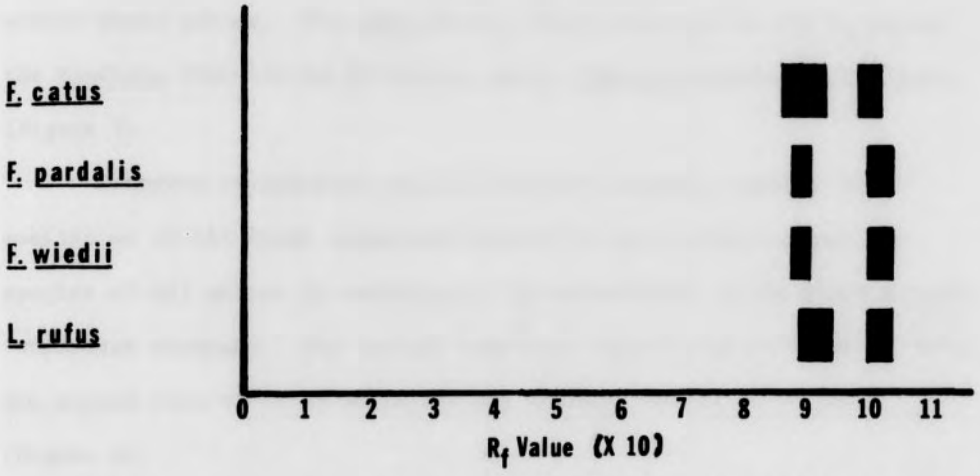

FIGURE 1. Representative electrophoretic patterns of hemoglobin from four felid species. R_f values are used in order to standardize the mobilities.

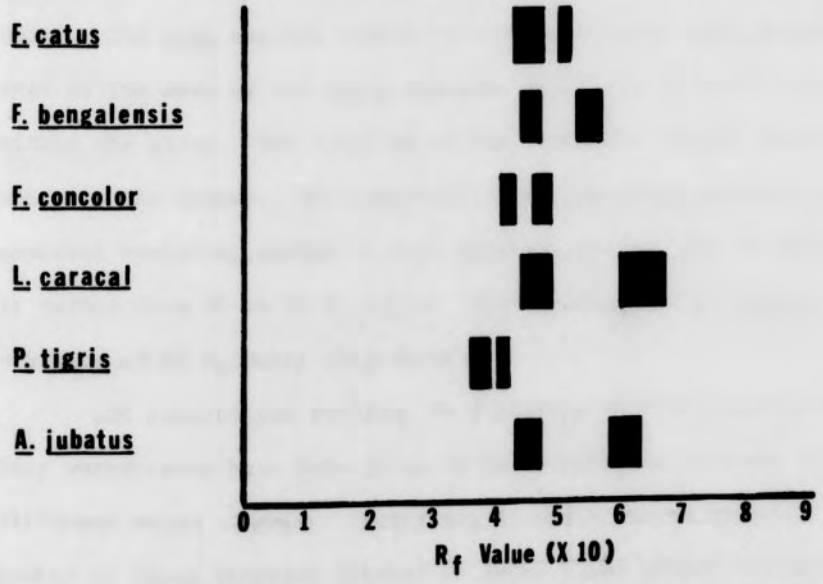

FIGURE 2. Representative electrophoretic patterns of the hemoglobin-haptoglobin complex from six felid species. R_f values are used in order to standardize the mobilities.

Felis species, the mobility of the leading edge of the component ranging from 109 to 126 R_f units. The remaining species showed little variability within their groups. The Lynx species varied from 110 to 117 R_f units, the Panthera from 114 to 121 units, and A. jubatus migrated at 120 units (Figure 3).

Esterase separations resulted in three distinct bands. The mobilities of all three components seemed to vary little between the species of all genera in relation to the variability in the other protein components examined. The fastest component ranged from 53 to 68 R_f units, the second from 41 to 53 units and the slowest from 28 to 39 units (Figure 4).

Ceruloplasmin migrated as a single band. There was a great deal of variability within the Felis species which ranged from 57 to 75 R_f units. The Lynx species seemed to have mobilities approximately equal to that of the mean of the Felis species, and there is little variability within the group. The mobility of the component ranged from 64 to 66 units in the lynxes. The component in the Panthera species, although somewhat variable, seemed to move slightly faster than in the Felis species. It varied from 70 to 82 R_f units. Ceruloplasmin in A. jubatus showed a mobility of 61 R_f units (Figure 5).

LDH separations resulted in a pattern of five homologous bands. Many vertebrates have been found to have different isozymes of LDH in different organ tissues. Therefore, it would not be uncommon to find a number of these isozymes present in felid blood plasma and serum. There was little variation in the migrational patterns of LDH either within or between suspected generic groups. The fastest band migrated over a range

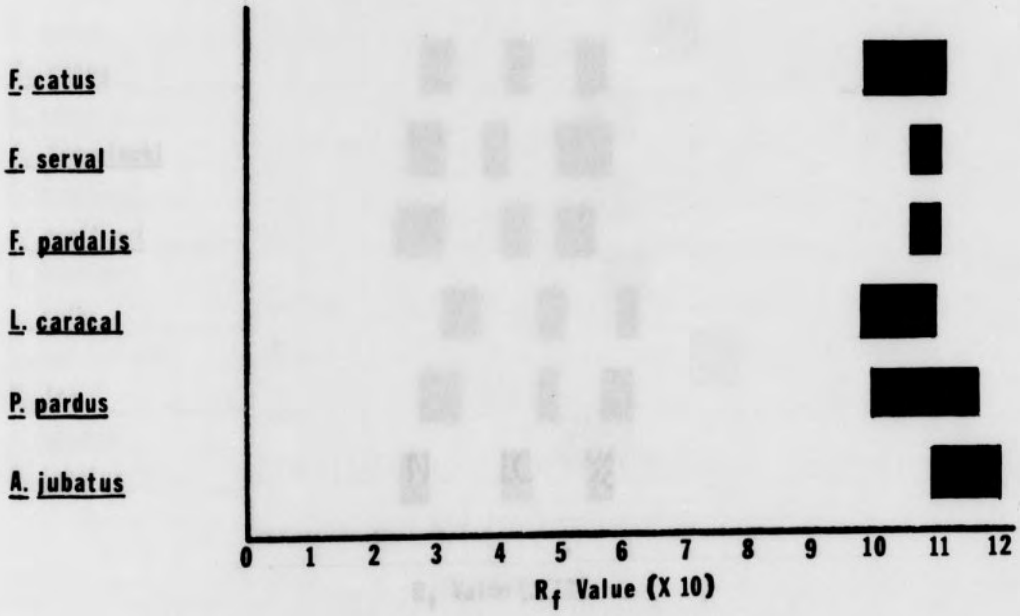

FIGURE 3. Representative electrophoretic patterns of albumin separations from six felid species. R_f values are used in order to standardize the mobilities.

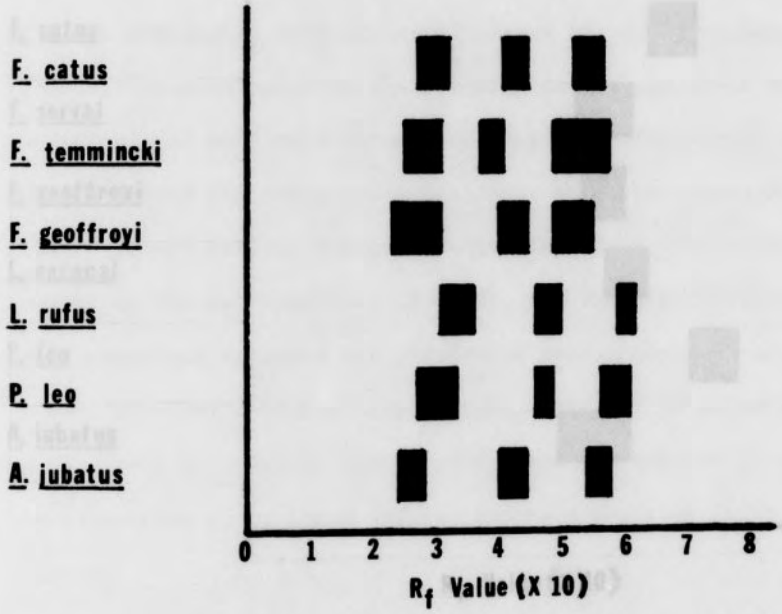

FIGURE 4. Representative electrophoretic patterns of esterase separations from six felid species. R_f values were used in order to standardize the mobilities.

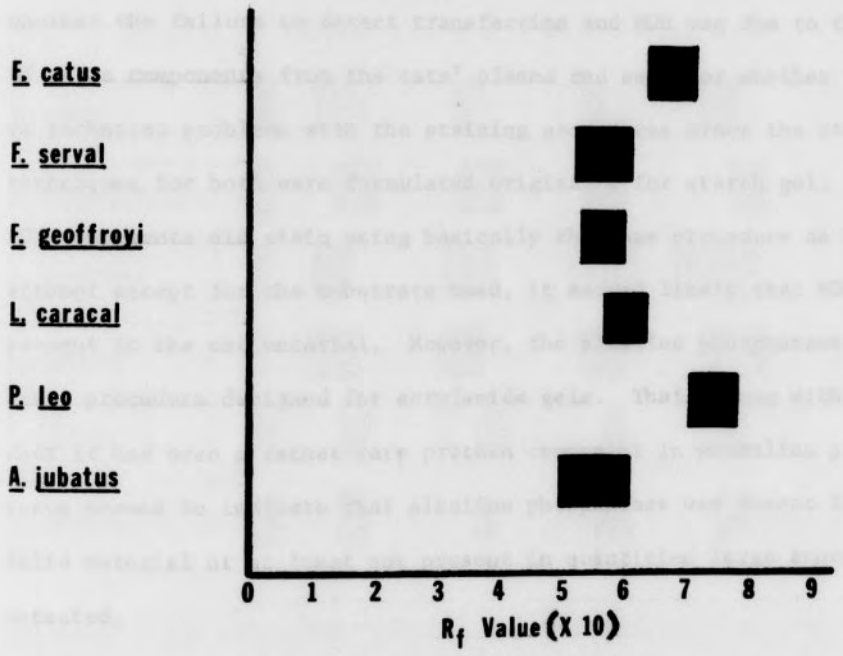

FIGURE 5. Representative electrophoretic patterns of ceruloplasmin separations from six felid species. R_f values were used in order to standardize the mobilities.

from 75 to 86 R_f units. The second band ranged from 53 to 64 R_f units. The range for the third band was from 35 to 45 units. The fourth band had a range from 14 to 20. The fifth band did not completely migrate from the origin (Figure 6).

No results were obtained from attempts to separate and stain alkaline phosphatase, transferrins or MDH. It was difficult to determine whether the failure to detect transferrins and MDH was due to the absence of those components from the cats' plasma and serum or whether it was due to technical problems with the staining procedures since the staining techniques for both were formulated originally for starch gel. Since the LDH components did stain using basically the same procedure as in the MDH attempt except for the substrate used, it seemed likely that MDH was not present in the cat material. However, the alkaline phosphatase technique was a procedure designed for acrylamide gels. That, along with the fact that it had been a rather rare protein component in mammalian plasma and serum seemed to indicate that alkaline phosphatase was absent from the felid material or at least not present in quantities large enough to be detected.

Data Analysis

In analyzing the data from the various electrophoretic patterns using multivariate discriminant analysis, it was determined that the packaged computer program could only use as test samples those which had measured values for each variable tested. However, there were cases in which the presence of a particular protein could not be detected in a particular individual even though it might be present in other individuals

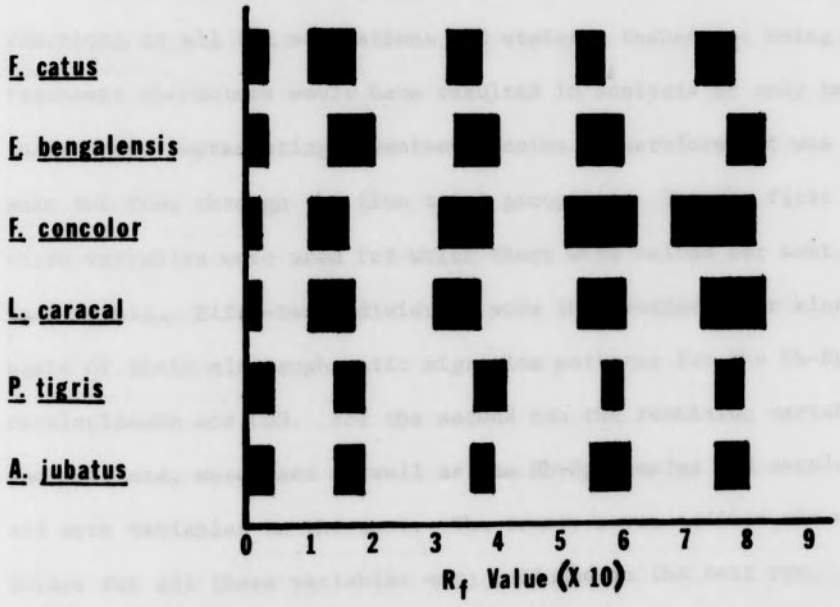

FIGURE 6. Representative electrophoretic patterns of LDH separations from six felid species. R_f values were used in order to standardize the mobilities.

of the same species. In one extreme case, F. wiedii, the sample lacked all proteins except Hb, the Hb-Hp complex, albumin and ceruloplasmin. Therefore, the individual was eliminated from the analysis, and F. wiedii was not represented in the analysis. In addition there were a few individuals which could not be tested for all proteins because there was not a sufficient amount of material available to run all the tests.

Limiting the analysis to only those individuals with positive reactions to all the separations and staining techniques being used as taxonomic characters would have resulted in analysis of only twenty-seven individuals representing seventeen species. Therefore, it was decided to make two runs through the five trial groupings. For the first run only those variables were used for which there were values for most of the individuals. Fifty-two individuals were thus analyzed for kinship on the basis of their electrophoretic migration patterns for the Hb-Hp complex, ceruloplasmin and LDH. For the second run the remaining variables, albumin and esterase, were used as well as the Hb-Hp complex and ceruloplasmin to add more variables to the test. The twenty-seven individuals which had values for all these variables were included in the test run. The group assignments resulting from the analysis of these variables have been given in the Appendix.

DISCUSSION

The grouping of individuals of seventeen felid species into possible genera by multivariate discriminant analysis of the electrophoretic patterns of five blood proteins has shown that the probability of the lion (leo), tiger (tigris) and leopard (pardus) being in the same genus Panthera and of the cheetah (jubatus) being the only species examined in the genus Acinonyx was great. However, the assignment of the remaining thirteen species to generic rank seemed less strongly indicated. (Note: An individual was placed in the group in which it had the highest probability of occurring in that particular set of groups. That is not to say that the group was the best of all possible groups in which the individual might be placed.)

The single cheetah individual tested was in both runs through the five trial groupings assigned to the genus Acinonyx. Only in the first run were any other individuals placed in the genus. One golden cat individual (F. temmincki) and one caracal individual (L. caracal) were consistantly assigned to that genus throughout run 1, and another golden cat individual was assigned to Acinonyx in the second and third trials. However, these two species were each represented by three additional individuals that were not placed in Acinonyx in any of the trials. This might be taken as evidence that there was some irregularity in the blood samples from the misplaced specimens or in the electrophoretic separation of one or more of the proteins examined.

The individuals assigned to the genus Panthera, the lion, tiger and leopard specimens, also seemed to be correctly allied. Only one individual from this group was assigned to another genus. This individual, a tiger, was allied with the genus Felis, more specifically the New World Felis, in trials I, II, IV and V of run 1. However, the same tiger individual, analyzed on the basis of a blood sample taken on a different date than the former, was included in the second run and was consistantly placed in Panthera. This, along with the seemingly illogical alliance of the tiger, an Old World great cat, with the smaller cats of the New World indicated that perhaps again there was some irregularity in the electrophoretic data on which the analysis of the misplaced tiger was based.

The placement of the smaller cat and lynx individuals was not as clear-cut as that of the great cats and the cheetah. In trials I and IV there seemed to be no real division between Felis and Lynx. Instead there was a good deal of overlap, with each group having individuals assigned to the other group. On the other hand, when the two were combined as in trial II, the new group seemed relatively cohesive. This would seem to indicate a rather close relationship between the lynxes and other smaller cats, although it appeared that there were some species that were more closely related to each other than to the rest of the species in the group. For example, from trials IV and V, it seemed that the smaller cats of the New World shared more similarities in the blood proteins examined with each other than with the Old World species. However, this division did not appear to be concrete enough to completely justify separate genera. Therefore, the lynxes and the other smaller cats examined seem more closely related to each other than to the other cat species and probably make up a single genus Felis.

The only possible exception to this grouping of the smaller cats could be that of the domestic cat (F. catus) and those species believed to have given rise to it (F. libyca and F. chaus). The first feline domestication was believed to have taken place in ancient Egypt in about 1600 B.C. Skulls of 190 cats found in Egyptian tombs dating from 600-200 B.C. have been identified as those of F. libyca and F. chaus. Therefore, these three species have been presumed to be very closely related (Ewer, 1973). This information assumed additional interest when it was noted that six of the fourteen individuals tested from these three species were consistantly grouped with the Panthera individuals; the remaining eight individuals were placed in Felis. Keeping in mind that individuals were placed in the group in which they had the highest probability of occurring of the groups given, one might hypothesize that this group of closely related species are different enough from all the other cats to be given separate generic rank. However, this hypothesis was not tested in this study. Therefore, additional individuals of these species should be investigated for their blood protein relationships and other taxonomic characters taken into consideration before any definite conclusions are drawn.

The differences in migration rates in electrophoretic patterns are assumed to be due to differences in the electrical charge of the protein molecules being compared. In addition, the size and shape of the molecules can affect the rate of migration. Therefore, the use of electrophoretic analysis of homologous proteins as a taxonomic character is based on the principle that divergent evolution would tend to give homologous proteins in more distantly related organisms different migration rates because of

changes in the amino acid structure and subsequent changes in net charge on the molecules.

However, it is not completely safe to assume the reverse of this; that is, homologous proteins with similar electrophoretic patterns may not be indicative of closely related organisms. There is, of course, the slight chance that two proteins that differ structurally may show similar migration patterns due to similarities in charge. However, electrophoretic coincidence, as this phenomenon is called, is not likely to be of concern if the basis for allying those species thought to be closely related is some criteria other than their electrophoretic character (Sibley, 1970). Such was the case in this study.

In addition, it must be recognized that evolutionary convergence onto a similar molecular arrangement may also be possible, especially in cases where life styles are quite similar and thus similar selective pressures may be operating. Although this problem might appear to be serious with the cats, all of which are carnivorous and are anatomically quite similar, the opposite is probably true. The cats are a widely distributed family geographically, and often the ranges of different species overlap. Therefore, it would seem logical that in a group of animals with many structural similarities as well as similarities in food preference, the stronger selective pressures would be those tending toward the divergence of the different felid species in order to reduce interspecific competition. Other reasons for the unlikelihood of this situation to cause problems in interpreting electrophoretic patterns of homologous proteins have also been given by Zuckerkandl and Pauling (1965) and Sibley (1970).

Thus, in reviewing the evidence presented herein, it seems evident that the genera Panthera and Acinonyx are relatively well-defined in terms of the species examined. The genera Felis and Lynx are not as easily defined. It does appear that they are more closely related to each other than to the remaining species tested and are perhaps closely enough related to be included in a single genus Felis. However, final decision on this alliance should probably be based on analysis of all four lynx species rather than only on the two examined in this study.

In addition, although the jaguar (P. onca) has usually been allied with the great cats, there has been some questioning of that alliance on the basis of its geographic isolation from the other great cats. The imperfect ossification of the hyoid in the jaguar was viewed by Pocock (1917) as conclusive evidence of its relationship to the remaining great cats. However, Leyhausen (1973) has viewed this character more as a function of size than of kinship. Therefore, the placement of the jaguar is unsure. Analysis of the electrophoretic patterns of jaguar blood proteins might indicate a closer relationship to the cats of the New World than to the Old World great cats, the hyoidal structure being due to convergent evolutionary forces or anatomical limitations rather than to close kinship with those species which have been determined to be included in the genus Panthera.

The snow leopard (Uncia uncia) and the clouded leopard (Neofelis nebulosa) also have not been examined electrophoretically. One or the other or both have been included together in the genus Panthera (Simpson, 1945; Anderson and Jones, 1967; and Ewer, 1973). Moreover, both have been classified as genera separable from each other and from Panthera (Allen,

1867, Pocock, 1917 and Walker et al., 1964). Therefore, electrophoretic examination of the blood proteins of these two species would be desirable as an indication of their rank.

As for the controversy over nomenclature of the two genera which include the great cats and the cheetah, there seems to be less to resolve. The conventions both of priority and of common usage would seem to indicate that Panthera Oken, 1816 be used as the generic name of the great cats rather than Leo Brehm, 1829 and that Acinonyx Brookes, 1829 be used as the generic name of the cheetah rather than Cynailurus Wagler, 1830 or Gueparda Gray, 1867. There seems to be no basis for the invalidation of Panthera by Anderson and Jones (1967).

The original question as to the generic distribution of the Felidae, to some extent, still remains unanswered. It seems reasonable to assume that the seventeen species examined for this study should be allocated to one of three genera; Felis, Panthera or Acinonyx. However, additional investigation is necessary to determine whether the remaining twenty-one species of cats can validly be included in only those three genera.

SUMMARY

Electrophoretic comparisons of a number of blood proteins were undertaken in order to clarify the generic relationships of the extant Felidae. The patterns of fifty-nine individuals, representing seventeen felid species, obtained from the haptoglobin-hemoglobin complex, albumin, esterases, ceruloplasmin and lactic dehydrogenase isozymes in acrylamide gel were analyzed using multivariate discriminant analysis. The results of the analysis seemed to indicate that the seventeen species examined might best be placed in three genera; Felis, Panthera and Acinonyx, with the lynxes included with the other smaller cats in Felis. Additional investigation is required, however, to determine whether all of the twenty-one species remaining untested could be included in those three genera or whether presently unrecognized relationships might not exist between some of those untested species and the species that were examined in this study.

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APPENDIX

Results of Multivariate Discriminant Analysis of Felid Electrophoretic Patterns

In all trials Run 1 is the analysis of the kinship of fifty-two individuals on the basis of their electrophoretic migrational patterns for the Hb-Hp complex, ceruloplasmin and LDH. In Run 2 twenty-seven individuals have been analyzed on the basis of the Hb-Hp complex, albumin, esterase and ceruloplasmin patterns.

Trial I

Run 1 (Analysis of 52 individuals)

Experimental Grouping	Computer Assignments				Total
	Felis	Lynx	Panthera	Acinonyx	
Felis	27	5	8	1	41
Lynx	3	1	0	1	5
Panthera	1	0	4	0	5
Acinonyx	0	0	0	1	1

Run 2 (Analysis of 27 individuals)

Experimental Grouping	Computer Assignments				Total
	Felis	Lynx	Panthera	Acinonyx	
Felis	14	3	2	0	19
Lynx	0	3	0	0	3
Panthera	0	0	4	0	4
Acinonyx	0	0	0	1	1

Trial II

Run 1 (Analysis of 52 individuals)

Experimental Grouping	Computer Assignments			Total
	Felis	Panthera	Acinonyx	
Felis	35	8	3	46
Panthera	1	4	0	5
Acinonyx	0	0	1	1

Run 2 (Analysis of 27 individuals)

Experimental Grouping	Computer Assignments			Total
	Felis	Panthera	Acinonyx	
Felis	20	2	0	22
Panthera	0	4	0	4
Acinonyx	0	0	1	1

Trial III

Run 1 (Analysis of 52 individuals)

Experimental Grouping	Computer Assignments		Total
	Felis	Acinonyx	
Felis	48	3	51
Acinonyx	0	1	1

Run 2 (Analysis of 27 individuals)

Experimental Grouping	Computer Assignments		Total
	Felis	Acinonyx	
Felis	26	0	26
Acinonyx	0	1	1

Trial IV

Run 1 (Analysis of 52 individuals)

Experimental Grouping	Computer Assignments: Old World Felis	New World Felis	Lynx	Panthera	Acinonyx	Total
Old World Felis	18	4	2	8	1	33
New World Felis	1	7	0	0	0	8
Lynx	2	1	1	0	1	5
Panthera	0	1	0	4	0	5
Acinonyx	0	0	0	0	1	1

Run 2 (Analysis of 27 individuals)

Experimental Grouping	Computer Assignments: Old World Felis	New World Felis	Lynx	Panthera	Acinonyx	Total
Old World Felis	9	2	1	2	0	14
New World Felis	1	4	0	0	0	5
Lynx	1	0	2	0	0	3
Panthera	0	0	0	4	0	4
Acinonyx	0	0	0	0	1	1

Trial V

Run 1 (Analysis of 52 individuals)

Experimental Grouping	Computer Assignments: Old World Felis + Lynx	New World Felis + Lynx	Panthera	Acinonyx	Total
Old World Felis + Lynx	21	6	8	2	37
New World Felis + Lynx	0	9	0	0	9

Run 1 (continued)

Experimental Grouping	Computer Assignments: Old World *Felis* + *Lynx*	New World *Felis* + *Lynx*	*Panthera*	*Acinonyx*	Total
Panthera	0	1	4	0	5
Acinonyx	0	0	0	1	1

Run 2 (Analysis of 27 individuals)

Experimental Grouping	Computer Assignments: Old World *Felis* + *Lynx*	New World *Felis* + *Lynx*	*Panthera*	*Acinonyx*	Total
Old World *Felis* + *Lynx*	12	3	1	0	16
New World *Felis* + *Lynx*	0	6	0	0	6
Panthera	0	0	4	0	4
Acinonyx	0	0	0	1	1