Differential rates of genic and chromosomal evolution in bats of the family Rhinolophidae

M. B. QUMSIYEH[1]
The Museum, P.O. Box 4499, Texas Tech University, Lubbock, TX, U.S.A. 79409
and
Department of Biological Sciences, Texas Tech University, Lubbock, TX, U.S.A. 79409
R. D. OWEN
The Museum, P.O. Box 4499, Texas Tech University, Lubbock, TX, U.S.A. 79409
AND
R. K. CHESSER
The Museum, P.O. Box 4499, Texas Tech University, Lubbock, TX, U.S.A. 79409
and
Department of Biological Sciences, Texas Tech University, Lubbock, TX, U.S.A. 79409

Corresponding Editor: R. Appels
Received June 18, 1987
Accepted January 14, 1988

QUMSIYEH, M. B., OWEN, R. D., and CHESSER, R. K. 1988. Differential rates of genic and chromosomal evolution in bats of the family Rhinolophidae. Genome, **30**: 326–335.

Data for nondifferentially stained chromosomes from 10 species of *Rhinolophus* (Chiroptera: Rhinolophidae) suggest a conserved chromosomal evolution. G-banded chromosomes for three well differentiated species (*Rhinolophus hipposideros*, *Rhinolophus blasii*, and *Rhinolophus acuminatus*) corroborate a low level of gross chromosomal rearrangements. Additionally, a comparison between G-banded chromosomes of *Rhinolophus* (Rhinolophidae) and *Hipposideros* (Hipposideridae) suggests extreme conservatism in chromosomal arms between these two distantly related groups. On the other hand, we report extensive genic divergence as assayed by starch gel electrophoresis among these 10 species, and between *Rhinolophus* and two hipposiderid genera (*Hipposideros* and *Aselliscus*). The present chromosomal data are not sufficient for phylogenetic analysis. Phylogenies based on electrophoretic data are in many aspects discordant with those based on the classical morphological criteria. Different (and as yet not clearly understood) evolutionary forces affecting chromosomal, morphologic, and electrophoretic variation may be the reason for the apparent lack of concordance in these independent data sets.

Key words: Rhinolophidae, chromosomes, electrophoresis, phylogeny.

QUMSIYEH, M. B., OWEN, R. D., et CHESSER, R. K. 1988. Differential rates of genic and chromosomal evolution in bats of the family Rhinolophidae. Genome, **30** : 326–335.

Des données sur la coloration non-différentielle des chromosomes de 10 espèces de *Rhinolophus* (Chiroptera: Rhinolophidae) suggèrent une évolution conservatrice des chromosomes. Les bandes G des chromosomes de trois espèces bien différenciées (*Rhinolophus hipposideros*, *Rhinolophus blasii* et *Rhinolophus acuminatus*) corroborent l'existence d'un faible niveau de réarrangements palpables des chromosomes. De plus, une comparaison entre les bandes G des chromosomes de *Rhinolophus* (Rhinolophidae) et d'*Hipposideros* (Hipposideridae) suggère un conservatisme extrême des bras chromosomiques entre ces deux groupes relativement distants. D'autre part, des électrophorèses sur gel d'amidon permettent de rapporter l'existence de différences géniques extensives entre ces 10 espèces, ainsi qu'entre *Rhinolophus* et deux genres d'Hipposideridae: *Hipposideros* et *Aselliscus*. Les présentes données chromosomiques sont insuffisantes pour les fins d'analyses phylogénétiques. Les phylogénies basées sur les données obtenues par électrophorèse basées sur les critères morphologiques. Ce manque apparent de concordance entre groupes de données indépendants pourrait bien relever de forces évolutives différentes, qui affectent la variation chromosomique, morphologique et électrophorétique.

Mots clés : Rhinolophidae, chromosomes, électrophorèse, phylogénie.

[Traduit par la revue]

Introduction

The family Rhinolophidae is comprised of a single Recent diverse genus, *Rhinolophus*, containing 69 species (Corbet and Hill 1980). Interest in relationships and taxonomy of rhinolophid bats began shortly after *Rhinolophus* was erected as a genus by Lacépède in 1799. Numerous papers were published in the 19th century (cited in Tate and Archbold 1939), but it was not until Andersen's monumental works on *Rhinolophus* in the early part of this century (1905*a*, 1905*b*, 1918) that this genus received critical and extensive evaluation. Since the works of Andersen, no major analysis of the relationships within *Rhinolophus* was attempted. Authors from Tate and Archbold (1939) to Hill and Yoshiyuki (1980) have either accepted Andersen's classification and (or) made minor changes in position of some taxa. All earlier studies of evolution and relationships of *Rhinolophus* were based on traditional taxonomic characters, primarily the shape of the noseleaf, the position of the third upper premolar, and overall size. The problems of relationships between taxa were compounded by discovery of extensive variation in body and skull

[1]Present address: Department of Pharmacology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN, U.S.A. 38101.

Printed in Canada / Imprimé au Canada

measurements and other morphological characters that were grossly underestimated in the classical studies using few specimens (Koopman 1965, Kock 1969).

The goals of the present study were threefold. First, we evaluated the utility of chromosomal banding for studying phylogeny and speciation in *Rhinolophus*. Second, we evaluated the use of electrophoresis as an alternative tool for studying and testing published hypotheses of morphological affinities in the genus *Rhinolophus*. Third, we evaluated the level of divergence in *Rhinolophus* in chromosomes and electrophoresis relative to the known morphological divergence.

Materials and methods

Specimens examined

Specimens were collected from natural populations either from caves (with hand nets) or by mist nets. Animals were sacrificed and tissues were obtained as described below. The following is a list of specimens examined with karyotype numbers (TK, Texas Tech University; NK, University of New Mexico; SP, Carnegie Museum of Natural History). *Rhinolophus acuminatus*: Thailand, Surat Thani Province, Muang Surat Thani District, Khao Tha Phet Nature Study Center, 0.5 km S, 2 km E Surat Thani (TK 21333, 21381); Tha Chang District, 15 km N, 23 km W Ban Marvan (TK 21371, 21420). *R. affinis*: Thailand, Uthithani Province, Lansak District, Huai Khai Khang Wildlife Station, Rhao-Nang-Rum Wildlife Research Station (TK 21213). *R. blasii*: Jordan, Northern Province, Jerash Refugee Camp (TK 25501-3); Jordan Valley, Tabget Fahl Ruins (TK 25605-9). *R. clivosus*: South Africa, Cape Province, Farm Leelykstaat, 3 km NE Marydale (SP 4006, 4008). *R. cornutus*: Japan, Honshu Island, Hiroshima Prefecture, Oni-no-iwaya cave, 8.5 km W Tojo (NK 6136, 6138, 6139); Tsushima Island, Nagasaki Prefecture, Nita (NK 6213). *R. darlingi*: South Africa, Cape Province, Farm Leelykstaat, 3 km NE Marydale (SP 4007, 4008). *R. ferrumequinum ferrumequinum*: Jordan, Northern Province, Dibbine National Forest (TK 25504-6, 25508-10, 25512, 25515, 25517, 25519-20, 25540-44). *R. f. nippon*: Japan, Honshu Island, Hiroshima Prefecture, Oni-no-iwaya cave, 8.5 km W Tojo (NK 6135); Japan, Tsushima Island, Nagasaki Prefecture, Nita (NK 6211-6212). *R. hipposideros*: Jordan, Northern Province, Dibbine National Forest (TK 25511, 25514). *R. malayanus*: Thailand, Uthithani Province, Lansak District, Tam Khe Nok, 3.6 km N, 2.6 km W Sanctuary Headquarters (TK 21260); Thailand, Chumphon Province, Pathiu District, 9 km N, 25 km E, Bar Nimit Charoen (TK 21457). *R. pusillus*: Thailand, Uthithani Province, Lansak District, Huai Kha Khang Wildlife Sanctuary (TK 21082). *Aselliscus tricuspidatus*: Papua New Guinea, East New Britain Province, 2 km S Gunanur (TK 20046). *Hipposideros diadema*: Papua New Guinea, Central Province, Apawawa Cave, 2 km ESE Jawarere (TK 20321). *H. armiger*: Thailand, Uthithani Province, Lansak District, 3.7 km S, 1 km E Khuo Nung-Rum Wildlife Reserve (TK 21190).

Chromosome studies

Nondifferentially stained chromosomal data were obtained from bone marrow preparations (Baker and Qumsiyeh 1988) and from previously published accounts. Bone marrow preparations proved inadequate for preparing G-banded chromosomes on rhinolophid bats. G-banded late prophase or early metaphase mitotic preparations were obtained from lung, ear, or embryonic tissue by methods described elsewhere (Baker and Qumsiyeh 1988). These latter techniques are very difficult especially considering the large number of chromosomes in species of *Rhinolophus*; therefore, data were obtained on only a fraction of the species attempted. Chromosomes were numbered according to size in *R. blasii* and then compared between species of *Rhinolophus* and finally to *Hipposideros*.

Electrophoresis

Heart, kidney, and liver tissues were collected and frozen in liquid nitrogen. These tissues were homogenized in an equal volume of grinding solution (1.21 g Trizma base, 0.37 g EDTA, pH 6.8, in 1 L H_2O). Tissue homogenates were centrifuged at 2000 rpm and stored at −80 degrees C. Horizontal starch gel electrophoresis and protein staining techniques were as described by Harris and Hopkinson (1977), and Selander et al. (1971). For enzymes with multiple isozymes, the presumed locus migrating most anodally was designated "1" and the others given increasing numbers respectively. For each locus, electromorphs were designated with their relative migration compared to the most common (designated 100 for anodal or −100 for cathodal) electromorphs. Twenty-six presumptive loci were consistently scorable and are listed below with their names and abbreviations as in Harris and Hopkinson (1977): aconitase (ACON); acid phosphatase (ACP); adenylate kinase (AK); albumin (ALB); catalase (CAT); creatin kinase (CK); esterase (ES); L-glutamate dehydrogenase (GLUD); glutamate oxaloacetate transaminase (GOT); α-glycerophosphate dehydrogenase (α-GPD); glucose phosphate isomerase (GPI-1,2); isocitrate dehydrogenase (IDH-1,2); lactate dehydrogenase (LDH-1,2); malate dehydrogenase (MDH-1,2); malic enzyme (ME); peptidase B (PEP-B); 6-phosphogluconate dehydrogenase (6PGD-1,2); phosphoglucomutase (PGM-1,2); sorbitol dehydrogenase (SORDH); transferrin (TRF).

Genetic distances (Rogers 1972) were calculated between each pair of species. Based on these distance values, three clustering methods were employed. The unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal 1973) is a phenetic method, with the clusters depicting overall similarity levels among taxa. Two phylogenetic methods also were employed, and, with our use of appropriate out-groups (Watrous and Wheeler 1981; Maddison et al. 1984), result in estimates of cladistic relationships of the *Rhinolophus* species examined. Two hipposiderid genera (*Aselliscus* and *Hipposideros*) were used as out-groups in these analyses. Although these taxa are genetically distant from the *Rhinolophus* species examined, one or both of them shares an electrophoretic character state with one or more *Rhinolophus* species in 9 of the 24 polymorphic loci examined. More importantly, the two out-group genera are members of the closest sister taxon to the group under examination.

The two phylogenetic methods are that of Fitch and Margoliash (1967), and the distance Wagner of Farris (1972). The Fitch-Margoliash method (FITCH) attempts to apportion homoplasy uniformly on the tree, whereas the Wagner method (WAGNER) is oriented only towards creating the shortest possible tree (maximum parsimomy), regardless of the location of any homoplasy. For the UPGMA analysis we used the NT-SYS program (Rohlf et al. 1979); for WAGNER we used the WAGNER78 program supplied by J.S. Farris; and for FITCH we used PHYLIP (Felsenstein 1982), employing the option in which negative branch lengths are not allowed on the tree.

To evaluate areas of congruence between the two phylogenetic estimates, we employed an Adams-2 consensus tree (Adams 1972; Rohlf et al. 1983). This method, which combines information from two trees into one, allows us to discern areas of congruence between the two estimates, and also depicts the level of resolution possible in areas of nonagreement. The consensus tree is the best estimate of the phylogeny of the *Rhinolophus* species examined, based on the electrophoretic data employed.

Results

Chromosomal study

The nondifferentially stained chromosomes of *Rhinolophus* show little variation in diploid and fundamental numbers. Table 1 summarizes the karyotypic data for the 10 species of *Rhinolophus* for which we studied electrophoretic variation. G-band data were available for *R. acuminatus*, *R. blasii* and *R. hipposideros*. Haploid complements of these three species are compared on a side-by-side basis in Fig. 1. The results show very few differences detectable between these species based on the quality of the G-bands available. Comparisons

TABLE 1. Karyotypic data for the examined species of *Rhinolophus*

Taxon	2*n*	fn	X	Y	References
R. acuminatus	62	60	ST	sM	Harada et al. 1982
R. affinis	62	69	ST	sM	Harada et al. 1985
R. blasii	58	60	1M/ST	sA	Dulić 1966; Qumsiyeh et al. 1986
R. clivosus	58	62	1A	sA	Dulić and Mutere 1974
R. cornutus	62	60	ST	ST	Ando and Uchida 1974
R. darlingi	58	60	ST	sST	Peterson and Nagorsen 1975
R. f. ferrumequinum	58	60	M	sA	Baker et al. 1975; Dulić 1966; Qumsiyeh et al. 1986
R. f. nippon	58	60	M	sA	Ando et al. 1983
R. hipposideros					
(Europe)	56	60	—	—	Capanna et al. 1967; Zima 1982
(Jordan)	58	60	SM	sA	Qumsiyeh et al. 1986
R. malayanus	62	60	ST	sM	Harada et al. 1982
R. pusillus	62	60	ST	sM	Harada et al. 1985

NOTE: A, acrocentric; M, metacentric; SM, submetric; ST, subtelocentric; s, small; l, large.

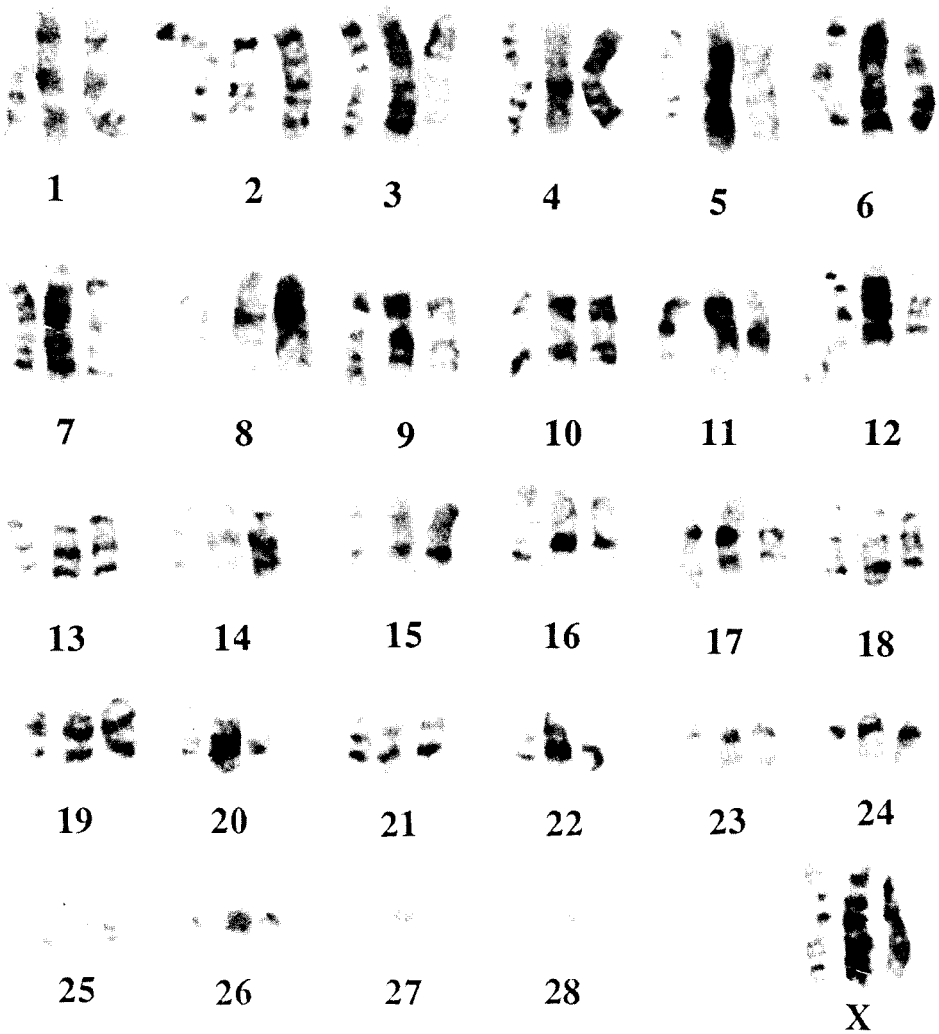

FIG. 1. Comparison of haploid complements from single cells of (left to right): *Rhinolophus acuminatus* (TK 21317), *R. blasii* (TK 25501), and *R. hipposideros* (TK 25511). *Rhinolophus acuminatus* differs from the other two species in chromosomes 12 (euchromatic addition), 15, and 16 (fissions). Homology for chromosomes smaller than chromosome 24 are tentative because of the small number of bands involved.

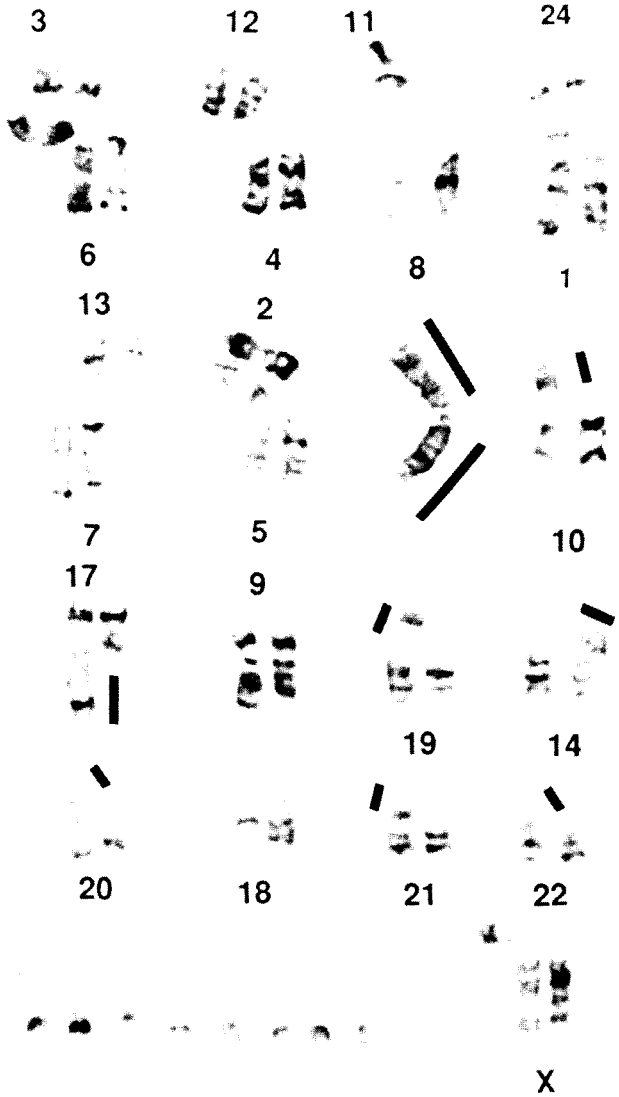

FIG. 2. Comparison of haploid complements of *Rhinolophus acuminatus* (TK 21317, acrocentric chromosomes) and *Hipposideros armiger* (TK 21190, biarmed chromosomes). Numbers as in Fig. 1. Bars represent unidentified homologs to the chromosomal arms of *Hipposideros*. Inset shows unmatched *R. acuminatus* autosomes.

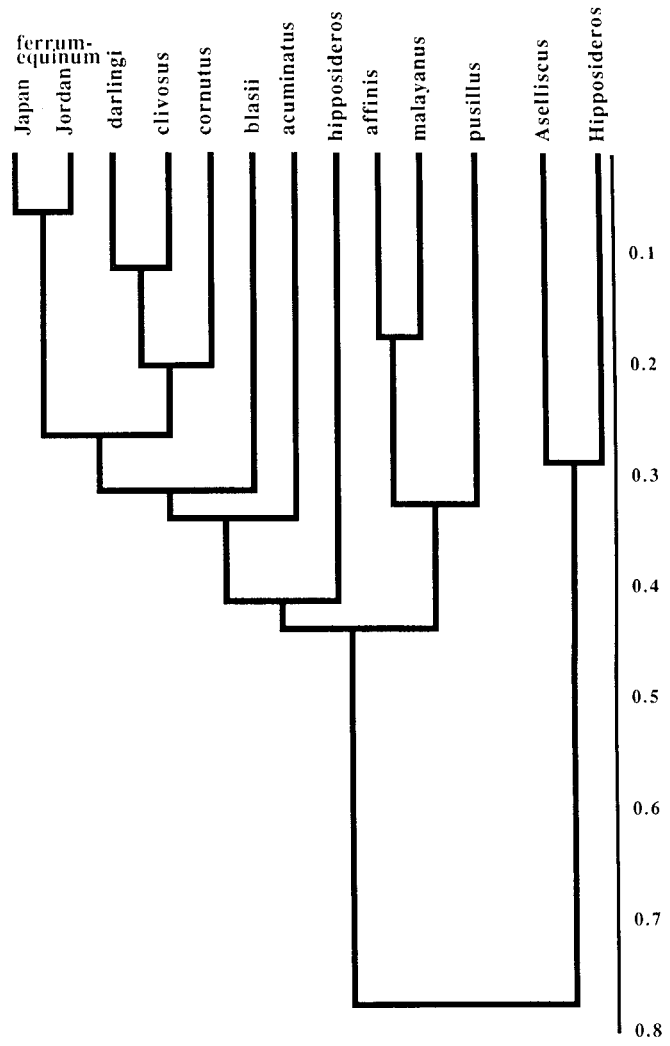

FIG. 3. UPGMA phenogram based on Roger's *D* distances among 11 rhinolophid and 2 hipposiderid taxa. Note the large intrageneric distances within *Rhinolophus*, and the extremely large distances between the two families. Cophenetic correlation coefficient is 0.97.

between *Rhinolophus* and *Hipposideros* (Fig. 2) show that homologous G-band sequences can be proposed for most of the chromosomal arms.

Electrophoretic study

Two of the 26 examined presumptive loci were monomorphic (GOT and LDH-2). All other loci showed variability in alleles in the different taxa examined (Appendix 1). The UPGMA phenogram (Fig. 3) represents intertaxon relationships based on overall similarities (as measured by Rogers' *D*). The phenogram has a cophenetic correlation coefficient of 0.97 and thus accurately represents the information contained in the intertaxon distance matrix. The two hipposiderids form a cluster separate from the *Rhinolophus* species examined. Within the rhinolophids, three general clusters are seen. One of these contains only *R. hipposideros*, which appears genetically quite distant from all examined congeners. *Rhinolophus affinis*, *R. malayanus*, and *R. pusillus* form a cluster. Within the remaining seven taxa, *R. acuminatus* and *R. blasii* are relatively distant from the others (clustering levels of 0.41 and 0.34, respectively). *Rhinolophus darlingi*, *R. clivosus*, and *R. cornutus* form a cluster, to which the two taxa of *R. ferrumequinum* are related at a clustering level of 0.25. The two *ferrumequinum* subspecies examined (*R. f. ferrumequinum* and *R. f. nippon*) have Rogers' *D* of 0.074.

The Fitch-Margoliash tree (Fig. 4A) shows *R. pusillus*, *R. affinis*, and *R. malayanus* forming a sister clade to the other *Rhinolophus* species examined. *Rhinolophus clivosus*, *R. darlingi*, and *R. cornutus* form the sister clade to *R. ferrumequinum*. Sister taxa to this group are *R. blasii*, *R. hipposideros*, and *R. acuminatus*, respectively.

The Wagner tree (Fig. 4B) also indicates that *Rhinolophus cornutus*, *R. clivosus*, and *R. darlingi* form a clade, with the two *R. ferrumequinum* subspecies being sister taxa to this group. To this clade, successive sister taxa are *R. blasii*, *R. hipposideros*, *R. acuminatus*, *R. affinis*, *R. malayanus*, and *R. pusillus*.

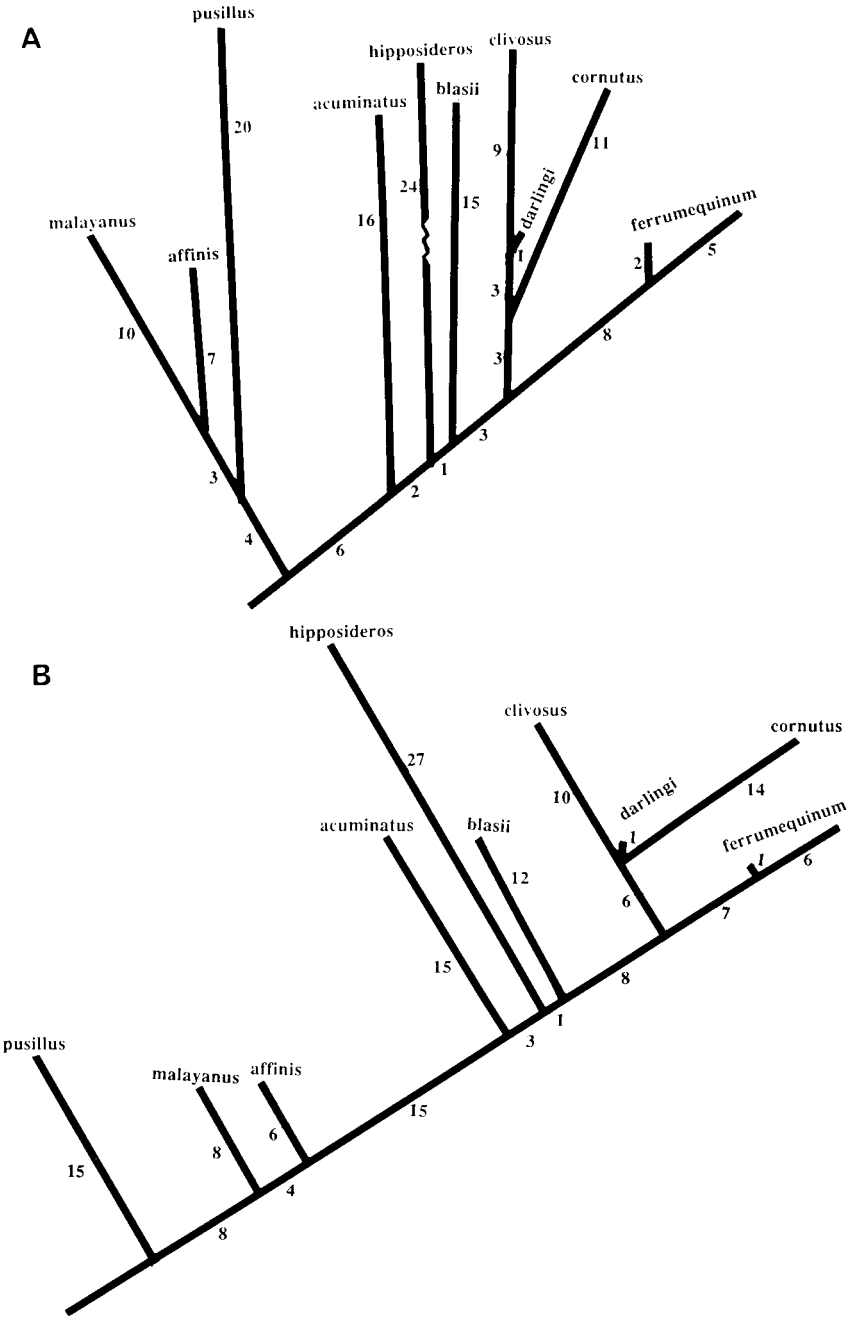

FIG. 4. Fitch-Margoliash (A) and Wagner (B) cladograms based on Rogers' distances among 11 rhinolophid taxa. Branch lengths on both trees are proportional to Rogers' D values ($\times$ 100) as they are distributed on the output trees. Trees are rooted in 2 hipposiderid taxa. Negative branch lengths were not allowed in the Fitch-Margoliash tree. The two trees agree on topology except for whether *R. malayanus*, *R. affinus*, and *R. pusillus* form a clade or merely a convex group. The two branches for *R. ferrumequinum* in this figure and Fig. 4B represent two analyzed populations from Japan and Jordan.

Discussion

Chromosomal evolution in Rhinolophus

Based on nondifferentially stained karyotypes, Harada et al. (1985) proposed that the genus be classified into three groups: the $2n = 62$, the $2n = 58$, and a third group with variable diploid numbers ($2n = 32-60$) "but with large biarmed autosomes." Most species of *Rhinolophus* fall into the first two groups, and only one species has a $2n = 32$ (Harada et al. 1985). However, it is now well documented that nondifferentially stained karyotypes are unreliable indicators of relationships (Haiduk et al. 1981; Qumsiyeh and Baker 1985; Baker et al. 1987). Additionally, *Rhinolophus hipposideros*, included in the third group because it has one pair of "large biarmed autosomes", has now been shown to have a different diploid number in Jordan ($2n = 58$ with no large biarmed autosomes) from those reported in Europe with $2n = 56$ (Qumsiyeh et al. 1986). Because of these reasons and the biological significance of this widespread genus, we decided to evaluate the use of G-banding techniques in studying evolution in *Rhinolophus*.

Because of the difficulties in acquiring G-band data on members of this genus, analyzable cytological preparations were obtained on only *R. acuminatus*, *R. blasii*, and *R. hipposi-*

deros. Comparison of these taxa indicated demonstrable G-band differences in only 3 of 28 euchromatic linkage groups. Chromosome 12 has an extra euchromatic segment and chromosomes 15 and 16 show fissions in *R. acuminatus*. Nondifferentially stained karyotypes for the genus *Rhinolophus* suggest that this pattern of few Robertsonian rearrangements differentiating taxa of *Rhinolophus* may be characteristic of the genus. This can be seen from the relatively constant number of autosomal arms (60−62) for most examined species of *Rhinolophus* even where the diploid numbers are different (Harada et al. 1985; Table 1). In this regard, *Rhinolophus* may prove to be similar to another ancient microchiropteran genus, *Myotis* (Bickham 1979; Bickham et al. 1986). The highly conserved nature of chromosomal evolution in this genus is further supported by the G-band homology with *Hipposideros* (Fig. 2). Many of the chromosomes of *Rhinolophus* show complete or almost complete G-band homology to chromosomal arms of *Hipposideros*. These chromosomes are (numbers as in Fig. 1): 1−14, 17−22, 24 and the X (Fig. 2). G-band homology between families of bats has been demonstrated for Vespertilionidae/Phyllostomidae (Bickham and Baker 1979) and Rhinopomatidae/Phyllostomidae/Pteropodidae (Qumsiyeh and Baker 1985). The addition of the Rhinolophidae/Hipposideridae comparison reported here further supports an early "canalization" of chromosomal evolution in bats (Bickham and Baker 1979). This model may not be applicable to other groups of mammals or to the cases of "karyotypic megaevolution" (see Baker et al. 1987 for a review). Additionally, if differences between species occur only by single or few Robertsonian rearrangements, then the present G-band data may not be useful in phylogenetic analyses (Qumsiyeh et al. 1987). The evidence from G-banding and studies by nondifferentially stained karyology suggests a limited role for karyotypic data in their present form in elucidating relationships and evolution of *Rhinolophus*.

Electrophoretic analyses

The low distance value (0.074) between the two populations of *R. ferrumequinum* is notable, given (*i*) the large geographic distance between the populations (Jordan and Japan), (*ii*) the Japanese forms have been considered as valid species by some authors due to their morphologic distinctness (Tate and Archbold 1939; Corbet and Hill 1980), and (*iii*) the fact that the Japanese bats are from island populations, a situation generally thought to contribute to genic divergence. Although additional data may prove otherwise, the high level of genic similarity between these conspecific populations, combined with the low level of variability in populations for which we have relatively large samples, suggests that even our smaller samples may accurately represent the species from which they were taken.

Buth (1984) discussed the desirability of using both isozyme and allozyme data in systematic studies. Among the *Rhinolophus* species examined here (as well as the two out-group genera) isozyme presences are identical, thereby restricting our study to the allozyme data. We opted to analyze an inter-taxon distance measure, rather than presence or absence of alleles, due to small size of a number of our study samples, combined with the large number of loci that we examined. In this methodology the sampling error for a particular allele in a given taxon is less likely to disproportionately affect the results than it would in construction of a cladogram by a locus-by-locus analysis. Use of distance data also avoids the problem of ordering the character states, a problem that may be solved only arbitrarily when two or more states occur in the in-group but not the out-group. We used Rogers' (1972) genetic distance because it is a metric statistic, a property that probably is particularly critical in phylogenetic estimation.

As pointed out by Swofford (1981), a UPGMA tree will reflect phylogeny only when the rates of evolutionary divergence are homogenous (equivalent in all clades). The UPGMA phenogram is topologically identical to the FITCH result except in the reversed placement of *R. acuminatus* and *R. hipposideros* on the trees. As this is an area in which the FITCH and WAGNER results are concordant, we suspect that a faster rate of electrophoretic divergence in *R. hipposideros* would best explain its more distant clustering rank in the phenogram. This interpretation must be viewed with some caution, however, due to the unusually large interspecific distances that characterize rhinolophid bats.

The WAGNER and FITCH phylogenies agree entirely except on the placement of *R. pusillus*, *R. malayanus*, and *R. affinis*. An Adams-2 consensus tree (Fig. 5B) recognizes the poor resolution in this portion of the tree, and suggests that the focal question concerns the placement of *R. pusillus*. In either case, *R. pusillus* appears to be a basal taxon that has become highly derived electrophoretically since divergence. The Adams-2 tree (Fig. 5B) retains the sister status of *R. malayanus* and *R. affinis* shown in the FITCH tree, and leaves this clade in an unresolved relationship with *R. pusillus* and the clade containing the remainder of the species examined. As shown by both the FITCH and WAGNER trees, *R. acuminatus*, *R. hipposideros*, and *R. blasii* evidently all arose from electrophoretically similar ancestors, and all (especially *R. hipposideros*) have evolved considerably since. Both *R. cornutus* and *R. clivosus* evidently arose from ancestors with protein complements much like the present-day *R. darlingi*, and these three species share a common ancestor with *R. ferrumequinum*.

Relationships and classifications of the genus Rhinolophus

It is constructive to compare the relationships of the examined taxa of *Rhinolophus* proposed by Andersen (1905*a*, 1905*b*, 1918) and the results of our electrophoretic analyses (Fig. 5). These two data sets show few similarities, such as the close association between *R. clivosus* and *R. darlingi* and between *R. f. ferrumequinum* and *R. f. nippon*. The lack of concordance between these data sets can be explained by one of the following hypotheses:

(*a*) The relationships based on classical taxonomic studies are inaccurate. This hypothesis is supported by (*i*) the extreme morphologic similarity of the taxa examined; (*ii*) the few morphologic characters analyzed, and (*iii*) that no clearly defined phylogenetic method was used.

(*b*) The relationships based on the electrophoretic study are inaccurate. This inaccuracy could be due to: (*i*) large genetic distances between taxa, a situation conducive to undetected convergence and reversal of electrophoretic character states; (*ii*) imprecisely estimating intraspecific variation due to small sample sizes; and (*iii*) electrophoretic data presented could not appropriately be analyzed for presence and (or) absence of alleles or direction of change (character state transformation).

(*c*) The results of morphologic and electrophoretic data may not be directly comparable at all levels in the phylogeny. Electrophoretic data may be useful for assessing relationships between closely related species and for subgeneric and generic relationships and morphologic data for higher taxonomic categories. Resolution at different phylogenetic levels by indepen-

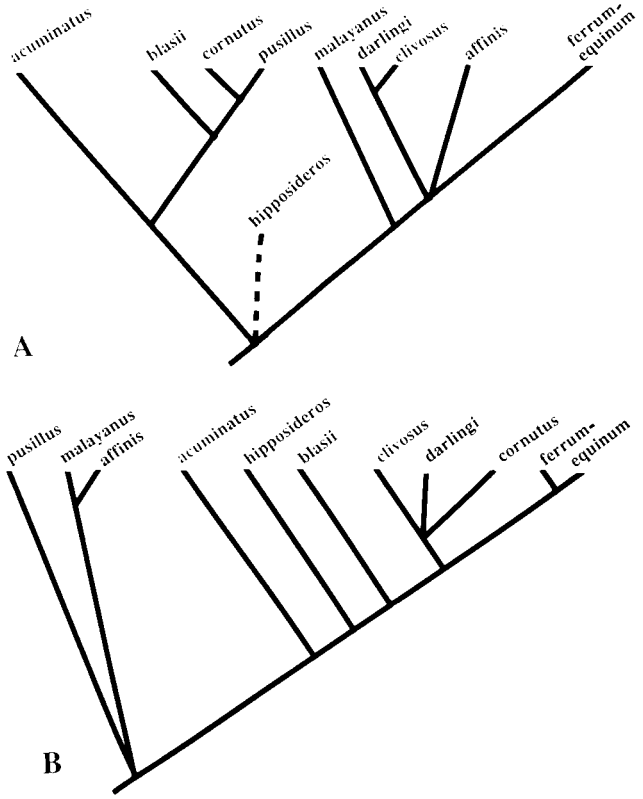

FIG. 5. (A) Summary of proposed phylogenetic relationships of rhinolophids examined here, based on Andersen (1905*a*). The position of *R. hipposideros* was not clearly elucidated. (B) Adams-2 consensus tree based on Fitch-Margoliash and Wagner parsimony trees in Fig. 4. This represents the best estimate of rhinolophid phylogeny, based on electrophoretic data examined here. Determination of branch lengths is not possible for a consensus tree.

dent data sets was shown by Arnold et al. (1982) in a study of phyllostomid bats. Possible methods for choosing between these alternatives could include studies using more molecular approaches (e.g., protein and DNA sequencing) or a reexamination of the classical morphological characters in a more rigorous way.

Regardless of use as phylogenetic markers, our studies of G-bands in 3 species and of electrophoresis in 10 species of *Rhinolophus* suggest extreme genic variability (as demonstrated by large genetic distances between species) accompanied by few demonstrable chromosomal differences. An example of this is that since diverging from a common ancestor, *R. hipposideros*, *R. blasii*, and *R. acuminatus* show as few as three demonstrable chromosomal changes but extensive electrophoretic divergence (Rogers' *D* values of 0.31 to 0.44, with several fixed differences [Appendix 1]). Similarly, the extremely large electrophoretic separation between *Rhinolophus* and *Hipposideros* ($D \cong 0.78$, Fig. 3) was apparently accompanied by mostly Robertsonian rearrangements with extreme conservation of linkage groups (Fig. 2). This situation can be explained by considering the history of *Rhinolophus*. *Rhinolophus* is an ancient genus with fossils recorded from late Eocene and early Oligocene in Europe (Dawson and Krishtalka 1984). Thus it is possible under both the neutral model (molecular clock) and, less so, the selection model to envision evolution of divergent electrophoretic patterns in *Rhinolophus*. Forces regulating chromosomal evolution are more difficult to discern, and debate over the various models of chromosomal evolution in mammals is far from resolved (Baker et al. 1987). To fully understand chromosomal evolution in Rhinolophoidae, high resolution G-band data and other genetic data such as gene mapping are needed for more species of both Rhinolophidae and Hipposideridae.

Acknowledgments

The specimens on which this study was based were collected on numerous field trips involving several institutions and many individuals, who cannot be listed because of space limitations. We thank those individuals and institutions that contributed by sponsoring, participating in field trips, donating equipment, providing logistical support, and issuing collecting permits. Special thanks are due to leaders of the field trips and their sponsoring institutions: Japan (Terry Yates, University of New Mexico), Papua New Guinea (James Dale Smith, University of California at Fullerton), South Africa and Thailand (Duane Schlitter, Carnegie Museum of Natural History), and Jordan (Texas Tech University). We thank Robert J. Baker for use of laboratory facilities for G-banding. Financial support for data analyses was provided by Texas Tech University and NSF grant DEB-83-00764 to R. J. Baker and R.K.C. We also appreciate the comments by Dr. Rudi Appels and two anonymous reviewers on an earlier draft of the manuscript.

ADAMS, E. N., III. 1972. Consensus techniques and the comparison of taxonomic trees. Syst. Zool. **21**: 390–397.

ANDERSEN, K. 1950*a*. On some bats of the genus, *Rhinolophus*, with remarks of their mutual affinities, and descriptions of twenty-six new forms. Proc. Zool. Soc. London II, 1905: 75–145.

——— 1905*b*. A list of the species and subspecies of the genus *Rhinolophus*, with some notes on their geographic distribution. Ann. Mag. Nat. Hist. (7) **16**: 648–662.

——— 1918. Diagnosis of new bats of the families Rhinolophidae and Megadermatidae. Ann. Mag. Nat. Hist. (9) **II**: 374–384.

ANDO, K., and UCHIDA, T. A. 1974. Karyotype analyis in chiroptera. 11. Phylogenetic relationships in the genus *Rhinolophus*. Sci. Bull. Fac. Agric. Kyushu Univ. **28**: 119–129.

ANDO, K., YASAZUMA, F., TAGAWA, T., and USHIDA, T. A. 1983. Further study on the karyotypic evolution of the genus *Rhinolophus* (Mammalia: Chiroptera). Caryologia, **36**: 101–111.

ARNOLD, M. L., HONEYCUTT, R. L., BAKER, R. J., SARICH, V. M., and JONES, J. K., JR. 1982. Resolving a phylogeny with multiple data sets: a systematic study of phyllostomid bats. Occas. Pap. Mus. Texas Tech Univ. **77**: 1–15.

BAKER, R. J., and QUMSIYEH, M. B. 1988. Methods in chiropteran mitotic chromosomal studies. *In* Ecological and behavioral methods for the study of bats. *Edited by* T. H. Kunz. Smithsonian Institution Press, Washington, DC. In press.

BAKER, R. J., DAVIS, B. L., JORDAN, R. J., and BINOUS, A. 1975. Karyotypic and morphometric studies of Tunisian mammals: bats. Mammalia, **38**: 697–710.

BAKER, R. J., QUMSIYEH, M. B., and HOOD, C. S. 1987. Role of chromosomal banding patterns in understanding mammalian evolution. *In* Current mammalogy, vol. 1. *Edited by* H. H. Genoways. Plenum Publishing Corp., New York. pp. 67–96.

BICKHAM, J. W. 1979. Banded karyotypes of 11 species of American bats (genus *Myotis*). Cytologia, **44**: 789–797.

BICKHAM, J. W., and BAKER, R. J. 1979. Canalization model of chromosomal evolution. *In* Models and methodologies in evolutionary theory. Bull. Carnegie Mus. Nat. Hist. **13**: 70–84.

BICKHAM, J. W., MCBEE, K., and SCHLITTER, D. A. 1986. Chromosomal variation among seven species of *Myotis* (Chiroptera: Ves-

pertilionidae). J. Mammal. **67**: 746–750.

BUTH, D. G. 1984. The application of electrophoretic data in systematic studies. Annu. Rev. Ecol. Syst. **15**: 501–522.

CAPANNA, F., CIVITELLI, M. V., and CORTI, L. 1967. I chromosomi somatici del pipistrello "Ferro di cavallo minore" (Mammalia – Chiroptera). Atti Accad. Naz. Lincei, U. Sci. Fis., Mat. Nat., Rend. **43**: 125–130.

CORBET, G. B., and HILL, J. E. 1980. A world list of mammalian species. British Museum (Natural History), London, Comstock.

DAWSON, M. R., and KRISHTALKA, L. 1984. Fossil history of the families of Recent mammals. *In* Orders and families of Recent mammals of the world. *Edited by* S. Anderson and J. K. Jones, Jr. Wiley Interscience, New York. pp. 11–57.

Dulić, B. 1966. Kromosomi somatickih stanica kao indikatori interspecificke srodnosti nekih Rinolofida (Mammalia, Chirotera). [Somatic chromosomes as indicators of interspecific relationships in certain rhinolophid bats.] (In Serbian, with French summary.) Biol. Glas. **19**: 65–96.

DULIĆ, B., and MUTERE, F. A. 1974. The chromosomes of two bats from East Africa: *Rhinolophus clivosus* Cretzschma 1928 and *Hipposiderus caffer* (Sundevall, 1946). Period. Biol. **76**: 31–34.

FARRIS, J. S. 1972. Estimating phylogenetic trees from distance matrices. Am. Nat. **106**: 645–668.

FELSENSTEIN, J. 1982. Package for inferring phylogenies (version 2.0). Published by the author, Seattle, WA.

FITCH, W. M., and MARGOLIASH, E. 1967. Construction of phylogenetic trees. Science, **155**: 279–284.

HAIDUK, M. W., BAKER, R. J., ROBBINS, L. W., and SCHLITTER, D. A. 1981. Chromosomal evolution in African Megachiroptera: G- and C-band assessment of the magnitude of change in similar standard karyotypes. Cytogenet. Cell. Genet. **29**: 221–232.

HARADA, M., MINEZAWA, M., TAKADA, S., YENBUTRA, S., NUNPAKDEE, P., and OHTANI, S. 1982. Karyological analyses of 12 species of bats from Thailand. Caryologia, **35**: 269–278.

HARADA, M., YENBUTRA, S., YOSIDA, T. H., and TAKADA, S. 1985. Cytogenetical study of Rhinolophus bats (Chiroptera, Mammalia) from Thailand. Proc. Jpn. Acad. Ser. B. **61**: 455–458.

HARRIS, H., and HOPKINSON, D. A. 1977. Handbook of electrophoresis in human genetics. North Holland Publishing Co., Amsterdam.

HILL, J. E., and YOSHIYUKI, M. 1980. A new species of *Rhinolophus* (Chiroptera, Rhinolophidae) from Iriomote Island, Ryuku Islands, with notes on the Asiatic members of the *Rhinolophus pusillus* group. Bull. Natl. Sci. Mus. Ser. A (Zool.) **6**: 179–189.

KOCK, D. 1969. Die Fledermaus-Fauna des Sudan. Abh. Senckenb. Naturforsch. Ges. **521**: 1–238.

KOOPMAN, K. F. 1965. Status of forms described or recorded by J. A. Allen in the American Museum Congo Expedition collection of bats. Am. Mus. Novit. **2219**: 1–34.

MADDISON, W. P., DONOGHUE, M. J., and MADDISON, D. R. 1984. Outgroup analysis and parsimony. Syst. Zool. **33**: 83–103.

PETERSON, R. L., and NAGORSEN, D. W. 1975. Chromosomes of fifteen species of bats (Chiroptera) from Kenya and Rhodesia. Life Sci. Occas. Pap. Royal Ont. Mus. **27**: 1–14.

QUMSIYEH, M. B., and BAKER, R. J. 1985. G- and C-banded karyotypes of the Rhinopomatidae (Microchiroptera). J. Mammal. **66**: 541–544.

QUMSIYEH, M. B., SCHLITTER, D. A., and DISI, A. M. 1986. New records and karyotypes of small mammals from Jordan. Z. Saeugetierkd. **51**: 139–146.

QUMSIYEH, M. B., HAMILTON, M. J., and SCHLITTER, D. A. 1987. Problems in using Robertsonian rearrangements in determining monophyly: examples from the genera *Tatera* and *Gerbillurus*. Cytogenet. Cell Genet. **44**: 198–208.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Tex. Publ. 7213: 145–153.

ROHLF, F. J., KISHPAUGH, J., and KIRK, D. 1979. NT-SYS. Numerical taxonomy system of multivariate statistical programs. Published by the authors, Stony Brook, New York.

ROHLF, F. J., COLLESS, D. H., and HART, G. 1983. Taxonomic congruence re-examined. Syst. Zool. **32**: 144–158.

SELANDER, R. K., SMITH, M. W., YANG, S. Y., JOHNSON, W. E., and GENTRY, J. B. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics, VI. Univ. Tex. Publ. 7103: 49–90.

Sneath, P. H. A., and SOKAL, R. R. 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.

SWOFFORD, D. L. 1981. On the utility of the distance Wagner procedure. *In* Advances in cladistics: proceedings of the first meeting of the Willi Hennig Society. *Edited by* V. A. Funk and D. R. Brooks. The New York Botanical Garden, Publications Office, Bronx, NY. pp. 25–43.

TATE, G. H. H., and ARCHBOLD, R. 1939. Results of the Archbold expeditions. No. 24. Oriental *Rhinolophus*, with special reference to material from the Archbold collections. Am. Mus. Novit. **1036**: 1–12.

WATROUS, L. E., and WHEELER, Q. D. 1981. The out-group comparison method of character analysis. Syst. Zool. **30**: 1–11.

ZIMA, J. 1982. [Karyotypes of three species of horseshoe bats from Czechoslovakia.] (In Czech with English summary.) Lynx (Prague), **21**: 121–124.

Appendix 1

Enzyme allele frequency data for 11 rhinolophid and 2 hipposiderid taxa examined. Taxa (and sample sizes): A, *Rhinolophus f. ferrumequinum* (16); B, *R. f. nippon* (13); C, *R. blasii* (8); D, *R. hipposideros* (2); E, *R. acuminatus* (4); F, *R. darlingi* (2); G, *R. clivosus* (2); H, *R. cornutus* (4); I, *R. affinis* (1); J, *R. pusillus* (1); K, *R. malayanus* (2); L, *Aselliscus tricuspidatus* (1); M, *Hipposideros diadema* (1).

Locus	Allele	A	B	C	D	E	F	G	H	I	J	K	L	M
ACON	−160						1.00	1.00	1.00					
	−120		0.33	0.62						1.00		1.00		
	−100	1.00	0.67	0.38		0.50					1.00			
	−90					0.50								
	−50				1.00									
	−20													1.00
	0												1.00	
ACP	200				0.25								1.00	
	150			1.00		1.00								
	100	1.00	1.00		0.75		1.00	1.00	1.00	1.00	1.00	1.00		1.00

Appendix 1 (*continued*)

Locus	Allele	A	B	C	D	E	F	G	H	I	J	K	L	M
AK	110				1.00	0.88				1.00	1.00	1.00	1.00	0.50
	100	1.00	1.00	1.00										
	75					0.12								
	70													0.50
	50						1.00	1.00	1.00					
CAT	105			0.62										
	100	1.00	1.00	0.38	1.00		1.00	1.00	1.00					
	50					1.00							1.00	
	40									1.00	1.00	1.00		
	0													1.00
CK	120						0.50							
	115							0.50						
	110			1.00	1.00	1.00	0.50	1.00	0.50					
	100	1.00	1.00											
	50									1.00	1.00	1.00	1.00	1.00
ES	150													1.00
	110											1.00		
	100	1.00	1.00	1.00	0.50		1.00	1.00						
	75					0.25								
	65					0.50				1.00				
	55				0.50	0.25			1.00		1.00			
	0												1.00	
GLUD	115												1.00	1.00
	105										1.00	1.00		
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
α-GPD	110	0.06		0.25										
	105											1.00		
	100	0.88	0.67	0.75	0.50	1.00	1.00	1.00	1.00	1.00	1.00			
	95												1.00	1.00
	50	0.06	0.33		0.50									
GPI-1	200												1.00	1.00
	100	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00		
	50				1.00									
GPI-2	−75												1.00	1.00
	−85				1.00									
	−95			0.88										
	−100	1.00	1.00	0.12		0.50	1.00	1.00	1.00					
	−105									1.00	1.00	1.00		
	−110					0.50								
IDH-1	200												1.00	1.00
	120				1.00									
	100	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IDH-2	−95				1.00									
	−100	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
	−105										1.00			
LDH-1	100	1.00	1.00	1.00	1.00		1.00	1.00		1.00		1.00		
	95					1.00								
	92								1.00					
	90										1.00			
	80												1.00	1.00
MDH-1	200												1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
MDH-2	−100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
	−105												1.00	
ME	110		1.00		1.00									
	105									1.00				
	102								0.50					
	100	1.00				1.00	1.00	1.00	0.50		1.00	1.00		
	60			1.00										
	50													1.00
	30												1.00	

Appendix 1 (*concluded*)

Locus	Allele	A	B	C	D	E	F	G	H	I	J	K	L	M
PEP-B	150				1.00									
	110								1.00		1.00			
	105					0.50				1.00		1.00		
	100	1.00	1.00			0.50	1.00	1.00						
	95			1.00										
	80												1.00	1.00
6PGD-1	400												1.00	1.00
	300											0.50		
	200			1.00	1.00	0.75	1.00	1.00	1.00					
	190					0.25				1.00	1.00	0.50		
	100	1.00	1.00											
6PGD-2	110												1.00	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00		
	50							0.25						
PGM-1	200										0.50			
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00		
	50												1.00	1.00
PGM-2	−50					0.12								
	−80							0.50						
	−90		0.33	0.12										
	−100	1.00	0.67	0.88	1.00	0.88	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00
SORDH	110					0.25								
	100	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00	1.00	1.00	1.00		
	95												1.00	1.00
ALB	110							1.00						
	100	1.00	1.00	1.00	1.00	0.75	1.00		1.00	1.00		1.00		
	80					0.25								
	75										1.00		1.00	1.00
TRF	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
	90												1.00	