

Chromosomal and Protein Evolution in Morphologically Similar Species of *Praomys sensu lato* (Rodentia, Muridae)

M. B. Qumsiyeh, S. W. King, J. Arroyo-Cabrales, I. R. Aggundey, D. A. Schlitter, R. J. Baker, and K. J. Morrow, Jr.

Evidence of extensive chromosomal evolution in a biologically and economically important group of African murids of the *Praomys/Mastomys* complex was provided by examination of G- and C-band chromosomal data on *P. coucha* ($2n = 32$), *P. fumatus* ($2n = 38$), *P. hildebrandti* ($2n = 32$), *P. jacksoni* ($2n = 28$), *P. misonnei* ($2n = 36$), and *P. cf. tullbergi* ($2n = 35$). A coding system was developed for the chromosomal characters, and analyses were performed by a computer program to find the shortest tree with a minimum of 35 autosomal rearrangements (pericentric inversions, complex translocations, centric fusions, centric fissions, tandem fusions, euchromatic additions, and heterochromatic additions). The resulting phylogenetic hypothesis differs from traditionally accepted hypotheses regarding this complex group of rodents. The cytogenetic data show that 1) there is no support for the dichotomy of *Mastomys/Praomys* previously based on morphology, 2) the $2n = 32$ species from eastern Africa (*P. hildebrandti*) is distinct from the $2n = 32$ species from southern Africa (*P. natalensis*), and 3) there is a close association between *P. jacksoni* and *P. cf. tullbergi*. Polyacrylamide gel electrophoresis of liver membrane proteins demonstrated few differences in protein mobilities between species and even fewer between individuals of the same species taken from different habitats and localities in Kenya. Monoclonal antibodies produced against liver proteins of one species and tested for reactivity to other species confirmed the evolutionary similarity of species of this complex. This immunologic approach may provide a robust data set for future phylogenetic studies of murid rodents. Species identification and evaluation of the relationships among these African murids should be easier to elucidate by using a combination of data sets such as morphology, chromosomes, and immunology.

From the Division of Genetics, University of Tennessee Medical School, Memphis (Qumsiyeh); the Department of Biochemistry, Texas Tech University Health Science Center, Lubbock (Qumsiyeh, King, and Morrow); the Department of Biological Sciences and the Museum, Texas Tech University, Lubbock (Qumsiyeh, Baker, and Arroyo-Cabrales); the Section of Mammals, Carnegie Museum of Natural History, Pittsburgh (Schlitter); and the National Museums of Kenya, Nairobi (Aggundey). Financial aid for various stages of his work was received from the Harrington Cancer Center (Amarillo), the M. Graham Netting Fund, the Cordelia Scaife May Charitable Trust, a Rea postdoctoral fellowship (to Qumsiyeh), NIH grant No. AIO 242 (to Dr. Robert Traub), NSF grant No. DEB-83-0784 (to Baker and Dr. R. K. Chesser), and CONACYT scholarship No. 49479 (to Arroyo-Cabrales). The authors thank Mike Hancock and the Biostatistics Department at St. Jude Children's Research Hospital for help with the analyses of the ELISA results and Christy Wright and Laura Janacek for reading the manuscript. They are also grateful to the Office of the President, the National Council on Science and Technology, and the Kenya Wildlife Conservation and Management Department for permits to work in Kenya. Address reprint requests to Dr. Qumsiyeh, Division of Genetics, Suite 3, University of Tennessee Medical School, 711 Jefferson Ave., Memphis, TN 38163.

Banding techniques are of major importance in understanding patterns of evolution of chromosomes and relationships among taxa that exhibit different chromosomal conditions. G-band homology has been shown to reflect genetic homology,^{19,30,32} and G-bands can be compared between individuals, populations, species, genera, and even higher taxonomic categories in mammals.⁵

Murid rodents are biologically interesting because they have undergone rapid evolutionary radiation and because cytogenetic differences are common between genera, species, and even populations of the same species. Studies of African *Aethomys*,⁶ Australian *Melomys* and related genera,⁷ and European *Mus*⁸ have shown that certain lineages undergo specific types of chromosomal rearrangements ("karyotypic orthoselection"³⁶). We studied six species of African Muridae belonging to the *Praomys/Mastomys* species complex,

an assemblage of morphologically similar species that occupy highly diverse habitats throughout sub-Saharan Africa.²⁵ Because of external and cranial similarities, the systematics of this group remains poorly understood, and many species have not been described.³⁵ Our findings document a pattern of chromosomal evolution associated with little morphological change during radiation into complex habitats.

We performed a preliminary analysis of variation in liver proteins (assayed by means of polyacrylamide gel electrophoresis and immunoreactivity), using some of the specimens studied cytogenetically to assess whether variation at a third level of biological organization would be consistent with the extensive chromosomal variation or the low level of gross morphological variation. We tested the potential use of mouse monoclonal antibodies (MAbs) in phylogenetic studies of these African murids. MAbs are usually specific

antibodies that are directed against epitopes on the immunizing antigen that differ from those of the host.¹⁶ Because differences in antigenicity arise as a result of mutations during the evolutionary process, we expected to be able to generate MABs that could distinguish proteins from different individuals, populations, species, or genera of murids.

Materials and Methods

Chromosomal Study

Bone marrow preparations were made in the field in Kenya, or the specimens were transported live to the laboratory in Lubbock, Texas, and karyotyped by the yeast stress method of Lee and Elder.²⁰ G-bands were obtained by the method of Seabright³¹ as modified by Baker and Qumsiyeh.⁴ C-bands were obtained by the method of Stefos and Arrighi³³ as modified by Baker and Qumsiyeh.⁴ For each specimen examined, a minimum of three G-banded chromosomal spreads were photographed and karyotyped; individual chromosomes were compared on a side-by-side basis. Koop et al.¹⁸ and Baker et al.⁶ identified at least 14 autosomal chromosomes that were shared between such diverse taxa as representatives of North American Sigmodontidae, Australian Muridae, and South African Muridae. We used their numbering system (based on the karyotype of *Peromyscus*¹¹) to facilitate comparison with the taxa we examined. Three chromosomes found in some of the murids we examined could not be directly related with the numbering system and were designated A, B, and C.

Abbreviations and symbols used in conjunction with the numbers and letters of particular chromosomes are as follows: CF = centric fusion; E+ = euchromatic addition; H+ = heterochromatic addition; PEI = pericentric inversion; TFU = tandem fusion; and TR = translocation. Abbreviations used for taxa were as follows: PFU = *Praomys fumatus*; PHI = *Praomys hildebrandti*; PCO = *Praomys coucha*; PMI = *Praomys misonnei*; PJA = *Praomys jacksoni*; and PTU = *Praomys cf. tullbergi*.

We performed cladistic analyses of the chromosomal data using the outgroup method.²⁸ As outgroup characteristics for *Praomys*, we used those which are shared with *Aethomys* as an immediate outgroup and the proposed primitive conditions for Sigmodontidae⁶ as more distant outgroups. The primitive conditions were coded as 0, with derived states coded with roman numerals.

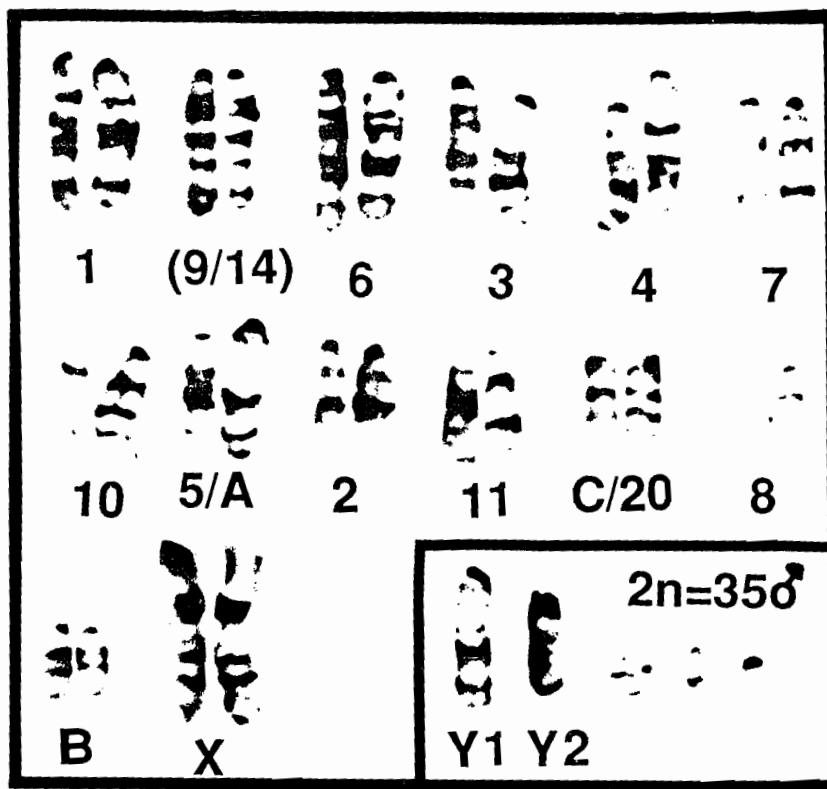


Figure 1. Comparison of haploid complements of *P. jacksoni* (TK 33552, left chromosome of each pair) and *P. cf. tullbergi* (TK 33554, right chromosome of each pair). Inset shows Y chromosomes and the remainder of the haploid autosomal complement of *P. cf. tullbergi*. Numbers refer to proposed homology to the *Peromyscus* standard numbering system.^{6,11} See Materials and Methods for abbreviations and symbols in this and subsequent figures.

Protein Purification, MAB Production, and Polyacrylamide Gel Electrophoresis

Livers were removed from dead animals and processed for karyotyping; 1 or 2 g of liver tissue was placed in a cryogenic tube in a liquid nitrogen tank for transfer to the laboratory. All additional storage was done at -20°C . Liver tissue was homogenized in TED buffer (10 mM Tris, 1 mM EDTA, 1 mM 1,4-dithiothreitol, pH 7.4), and the homogenate was centrifuged at 35,000 g for 30 min. The supernatant was discarded, and the pellet was resuspended in 3 M KCl and mixed gently overnight at 4°C . The mixture was then centrifuged again at 35,000 g for 60 min. The supernatant was mixed with an equal volume of saturated ammonium sulfate for 30 min, and the insoluble fraction was removed by centrifugation; the pellet was resuspended and dialyzed extensively in phosphate-buffered saline. Protein concentration was assayed by the Bradford method. Immunization and production of MABs was performed as described previously.^{10,12} Briefly, 100 μg of antigens was used to immunize *Balb/c* mice. After several immunizations, animals that showed evidence

of polyclonal antiserum were given a final booster injection 72 h before killing and spleen removal. Disaggregated and washed spleen cells were fused with twice as many mouse plasmacytoma cells (x63/Ag 8.653). Hybridomas were screened by enzyme-linked immunosorbent assay (ELISA) as described elsewhere,¹⁰ and those giving a positive reaction to the immunizing antigen were cloned at least twice in 96-well plates with mouse macrophages as feeder layers. At each stage of cloning and expansion, we selected the most stable and productive hybridomas to grow and to freeze as samples in liquid nitrogen.

The color intensity was read at 492 nm with an automated ELISA plate reader (Flow Laboratories).¹⁰ The absorbance reading for each well was recorded and corrected for background readings in control wells. This matrix of antibody-antigen reactivity provided the raw data for statistical analyses. Five separate sets of experiments—and thus five matrices—were performed to correct for experimental variance, resulting from factors other than affinity of MABs to antigens. The readings from each matrix were transformed to rank values (highest reactivity to lowest) and

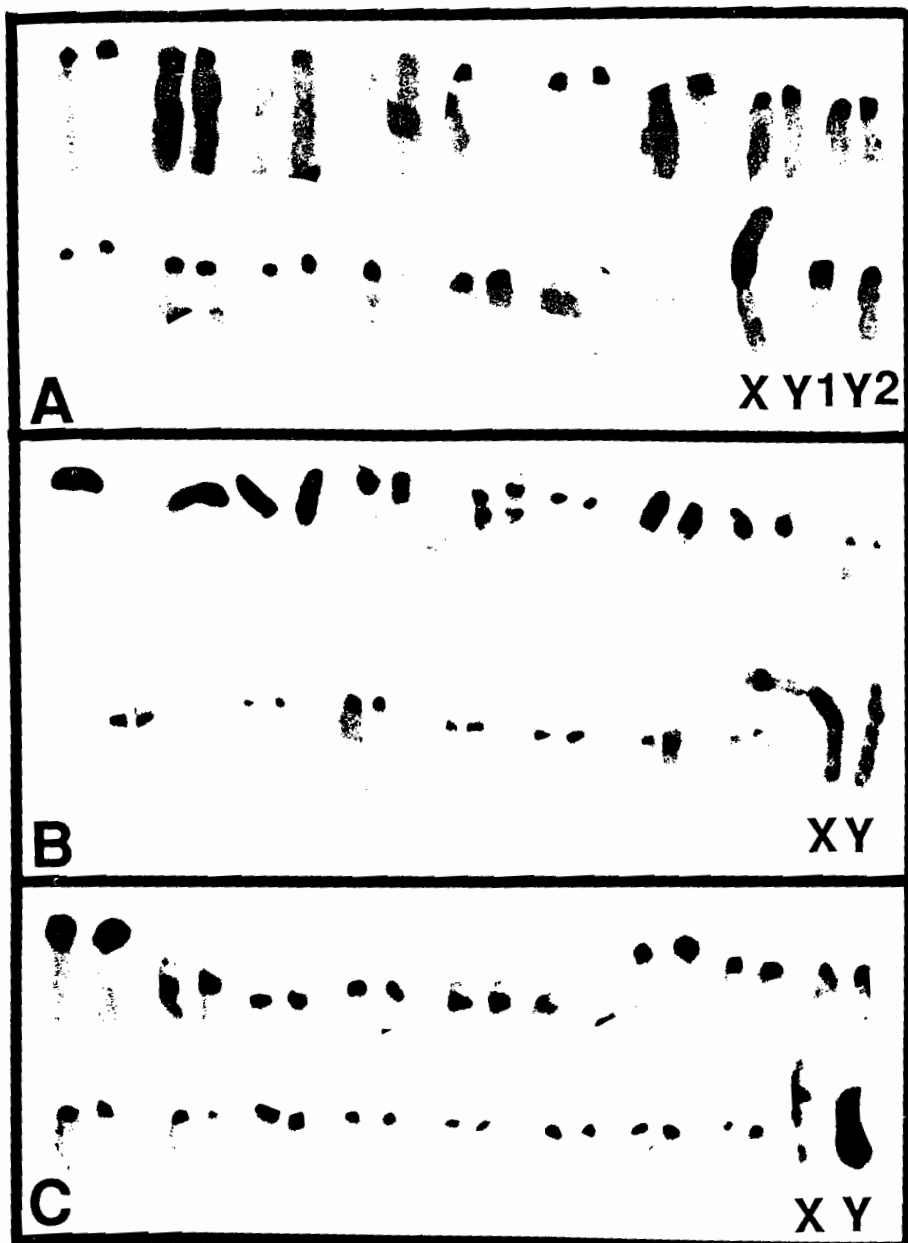


Figure 3. C-banded chromosomal complements of (A) *P. cf. tullbergi*, (B) *P. huberti*, and (C) *P. misonnei*.

and in such murids as Indo-Australian *Rattus* and are assumed to be primitive.⁶ These conditions were coded 3⁰ and 4⁰. The fusion of chromosomes 3 and 4 in PFU, PHI, and PMI is thus a derived condition for these taxa (conditions 3^I and 4^I). PMI has a further translocation on the fused meta-centric chromosome 3 and 4 (3^{II} and 4^{II}). The direction in this case is 3 or 4⁰-I-II. In other cases, we were able to postulate that all derived rearrangements are a single step away from the primitive condition (i.e., 0→I, 0→II, 0→III). After we coded character states and performed the transformation (see Appendix), the matrix was analyzed using the McClade computer

program (version 2.1, written by Wayne Maddison and David Maddison, Harvard University). We then used branch swapping to arrive at the most parsimonious tree representing a phylogenetic hypothesis of the relationships among these taxa (Figure 4).

Protein Data

Immunization of *Balb/c* mice with antigens representing membrane proteins from liver proteins of *P. fumatus* resulted in the production of polyclonal and monoclonal antibodies. This fact demonstrates that these antigens are different from the ho-

mologous mouse antigens and thus were considered as "foreign" proteins by the immunized mice. We selected seven MABs for further cloning and testing because of the stability of the hybridomas producing them and because they had strong reactivity to the immunizing antigen (Table 1).

Antigens from various individuals within a species reacted similarly to the seven MABs (data not shown). As expected, these antibodies reacted very strongly with the immunizing antigens from *P. fumatus*. Although antigens from various species were recognized by these MABs, there was variation not only in the reactions of each MAB to antigens from other species but also between the reaction of different MABs to the same antigen source (Table 1). Three of the seven MABs showed no specificity in their reaction to different antigens. With three MABs, PJA and PMI consistently reacted more weakly; thus, they were significantly different from the other antigens, including PFU, PCO, and PHI. Finally, MAB 2K4A6 consistently produced weaker reactions in PJA than did the immunizing antigen from PFU. *P. jacksoni* showed the lowest reactivity to MABs raised against *P. fumatus*, and this corresponds to the earlier branching of *P. jacksoni* on the chromosomal tree (Figure 4; *P. tullbergi* was not analyzed immunologically; see Discussion).

As with ELISA experiments, the SDS-PAGE results showed no differences between individuals of the same species taken from different localities. We performed this analysis on *P. hildebrandti* because we had specimens of this species representing different habitats and localities. PAGE of the extracted proteins showed no demonstrable gross differences in electrophoretic mobility between specimens from different localities (data not shown). Between species, only minor changes in mobility were noticeable; the most divergent patterns of PAGE were between *P. fumatus* and *P. jacksoni* (Figure 5). These differences can be explained by mobility shifts (changes in molecular weight) without assuming a differential protein content.

Discussion

In our study, using New World sigmodontid rodents as distant outgroups and *Aethomys* as a closer outgroup, we were able to determine the derived chromosomal conditions in the six taxa of *Praomys* we examined and the direction of chromosomal rearrangements (Figure 4 and Appendix). In constructing a phylogenetic

Table 1. Summary of five sets of ELISA experiments for reaction of antibodies raised to *P. fumatus* to antigens derived from various species of *Praomys* (species and specimen number).

MAb	Antigen source				
	<i>P. fumatus</i> 33112	<i>P. coucha</i> 25715	<i>P. hildebrandti</i> 33104	<i>P. misonnei</i> 33506	<i>P. jacksoni</i> 33475
2K2H11	23.0	19.6	15.5	11.5	13.1
2K2F9	32.8	27.4	26.4	22.6	21.2
2K1F3	27.6	24.5	20.2	25.1	18.2
2K2C7	30.8	24.9	22.3	19.6	19.6
2K5G9	20.4	14.4	14.1	13.8	11.0
2K4A6	20.5	9.0	13.9	12.4	7.0
2K3H8	12.1	12.6	5.6	8.3	8.9

For each experiment, antibody-antigen reactions were ranked from highest (35) to lowest (1), and average ranks were determined and are listed here. The lines beneath the average ranks indicate where the antigens of the different species show no significant difference in rank as assayed by the LSD test.³⁷

of relationships among the taxa of the *Praomys/Mastomys* complex in Africa, correlating new chromosomal data with known classifications has been difficult.^{9,13,14,17} Our knowledge of these rodents continues to expand with the incorporation of new cytogenetic evidence. In discussing what is known about karyotypic and taxonomic relationships, we use the generic name *Praomys sensu lato* because there is no cytogenetic evidence (and questionable morphological evidence) as to the validity of *Myomyscus* and *Mastomys* (Figure 4).

In southern Africa, two morphologically similar species with $2n = 32$ and $2n = 36$ occur sympatrically in some areas; these species are now referred to as *P. natalensis* and *P. coucha*, respectively.^{13,14} Individuals with $2n = 32$ also occur in western and eastern Africa. Those from Somalia with $2n = 32$ have a G-band pattern distinct from those of *P. natalensis* and are referred to as *P. huberti*, described originally from Nigeria.⁹ Comparison of the G-band data of specimens from Kenya with $2n = 32$ (Figure 2) with published G-bands on the South African form with the same diploid number¹⁴ confirmed the presence of an eastern African species distinct from *P. natalensis*. We use the name *P. hildebrandti* (Peters, 1878) originally described from southeastern Kenya because it predates *P. huberti* (Wroughton, 1908). It would be interesting to determine the status of the $2n = 32$ form of western Africa (*P. hildebrandti*, *P. natalensis*, or a distinct third species).

The $2n = 36$ form from Kenya was morphologically similar to the *P. jacksoni/tullbergi* complex. This form recently was described as a new species, *P. misonnei*.³⁵ Its chromosomal characteristics are very distinct and indicate that it is phylogenetically closer to *P. hildebrandti* than to *P. jacksoni* (Figure 4). Matthey²² reported an

all-acrocentric $2n = 34$ for *P. tullbergi* from the Ivory Coast. One of our specimens with $2n = 35$ (all acrocentric) obtained from the Kakamega Forest was morphologically and chromosomally similar to *P. jacksoni* ($2n = 28$), which was obtained from the same forest. This specimen is not referable to any of the named forms of this complex and may represent a new taxon. Pending collection of additional specimens and a reevaluation of the morphology of this complex group, we use the name *P. cf. tullbergi* for this taxon.

Proteins extracted from plasma membranes of various species of this African group of murids appear to be conserved. The two most divergent patterns we obtained were those between *P. fumatus* and *P. jacksoni*, with only four proteins showing consistent mobility shifts (Figure 5) and reactivity to four of the MAbs (Table 1). Conservation in structural protein patterns has been reported by two-dimensional gel electrophoresis of proteins from other rodent species.^{2,38} Therefore, some authors have used polyclonal antisera to study the evolutionary change of proteins. However, the polyclonal response to a certain protein can also change significantly as a result of a single amino acid substitution that alters the three-dimensional structure of the protein, exposing and/or hiding immunogenic epitopes.^{3,15} These difficulties can be avoided by using MAbs, which should bind to specific epitopes.

Our data suggest the need for further studies utilizing newer approaches in conjunction with the classical morphological studies in order to gain an understanding of the evolutionary history and to develop means of efficient species identification in African murids. Production of MAbs is tedious, but once they are produced, the hybridomas offer unique advantages: 1) MAbs

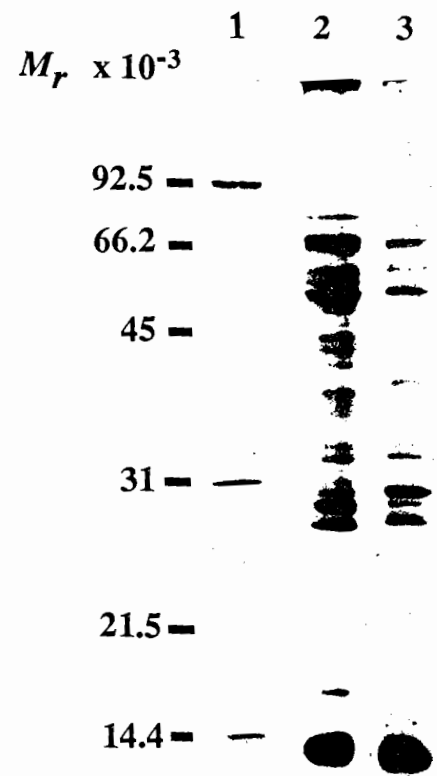


Figure 5. SDS-PAGE of liver proteins of *P. fumatus* (TK 33112, lane 2) and *P. jacksoni* (TK 33475, lane 3). Lane 1 represents molecular weight markers (values on left). Notice that differences between the two sets of proteins can be explained by mobility shifts and that no major deletions or additions need to be postulated.

provide a continuous supply of uniform and specific antibodies, 2) antigens of many species can be screened quickly for their reaction to the MAbs in one or a few ELISA tests, and 3) differences in reactivity to a particular MAb reflect change at a single epitope and thus can be subjected to rigorous cladistic analysis.¹⁶

In a study of the South African *Mastomys* (*Praomys*), Green et al.^{13,14} discussed the two karyotypic forms ($2n = 32$ and 36) as representing two cryptic species. Our data further illustrate the inadequacy of classical morphological studies in documenting variation in African *Praomys*. In one locality in Kenya (Western Province, Kakamega forest), we were able to characterize three distinct species of *Praomys* (PJA, PMI, and PTU) that were distinguished by many chromosomal rearrangements but were so similar phenotypically that we could not distinguish these taxa upon capture in the field.

Finally, we suggest that the *Praomys* complex itself might provide a rich source of experimental models potentially as use-

- and Setzer HW, eds). Washington, D.C.: Smithsonian Institution Press; 1974:1-39.
26. Petter F. Les *Praomys* d'Afrique Central. Z Säugetierk 1964; 30:54-60.
27. Qumsiyeh MB. Chromosomal fissions and phylogenetic hypotheses: cytogenetic and allozymic variation between species of *Meriones* (Rodentia, Gerbillidae). Occasional Papers Museum Texas Tech University, Lubbock 1989; 132:1-16.
28. Qumsiyeh MB, and Baker RJ. Comparative cytogenetics and the determination of primitive karyotypes. Cytogenet Cell Genet 1988; 47:100-103.
29. Qumsiyeh MB, Hamilton MJ, and Schlitter DA. Problems of using Robertsonian rearrangements in determining monophyly: examples from the genera *Tatera* and *Gerbillurus*. Cytogenet Cell Genet 1987; 44:198-208.
30. Sawyer JR, and Hozier JC. High resolution of mouse chromosomes: banding conservation between man and mouse. Science 1986; 232:1632-1635.
31. Seabright M. A rapid banding technique for human chromosomes. Lancet 1971; ii:971-972.
32. Stallings RL, Munk AC, Longmire JL, Jett JH, Wilder ME, Siciliano MJ, Adair GM, and Crawford BD. Oncogenes and linkage groups: conservation during mammalian chromosome evolution. Chromosoma 1985; 92: 156-163.
33. Stefos K, and Arrighi FE. Heterochromatic nature of the W chromosome in birds. Exp Cell Res 1971; 68: 228-231.
34. Tranier M. Parente des *Mastomys* du Maroc et du Senegal (Rongeurs, Murides). Mammalia 1975; 38:558-560.
35. Van der Straeten E, and Dieterlen F. *Praomys misonnei*, a new species of Muridae from Eastern Zaire (Mammalia). Stuttgart Beitr Naturk Ser A 1987; 402:1-11.
36. White MJD. Modes of speciation. San Francisco, California: Freeman; 1978.
37. Wiener BJ. Statistical principles in experimental design. New York: McGraw-Hill; 1971. 198 p.
38. Zimny-Arndt U, and Klese J. Qualitative and quantitative variability in different classes of proteins: comparison of mouse and rat. J Mol Evol 1987; 24:260-271.