Karyology and Chromosomal Evolution of Some Small Mammals Inhabiting the Rainforest of the Rabi Oil Field, Gabon

Ashley PRIMUS[1], Jessica HARVEY[1], Sylvain GUIMONDOU[2], Serge MBOUMBA[3], Raphaël NGANGUI[4], Federico HOFFMANN[5, 6], Robert BAKER[5] and Calvin A. PORTER[5, 1]

1 Introduction

The genetic information that living things inherit from their progenitors is encoded in the molecular sequences of DNA. The DNA of animals and other eukaryotes is combined with proteins and organized into chromosomes. Chromosomes are essential for the processes of cell division during mitosis and meiosis, which distributes genetic information to daughter cells. The process of evolution often produces changes in the shape, number, or size of the chromosomes. The karyotype of an individual is the chromosomal complement of its cells. The karyotype is often uniform within a species. However, in many cases, there is considerable karyotypic variation among the populations or individuals of a species. Comparison of karyotypes can provide valuable insight into relationships among species.

A substantial literature has been published on karyotypes of small African mammals (e.g., Robbins and Baker 1978, Haiduk *et al*. 1980, 1981; Viegas-Péquignot *et al*. 1983, Reumer and Meylan 1986, Zima *et al*. 1998, Schlitter *et al*. 1999, Biltueva *et al*. 2001). However, the region has a high level of biological diversity, and many species have never been studied karyotypically. For example, the shrew genus *Crocidura* contains more than 150 species, of which approximately 100 occur in Africa. Only about half of the African species have been karyotyped, and a considerable amount of karyotypic variation has been described, with diploid numbers in African *Crocidura* ranging from 36 to 68 (Zima *et al*. 1998, Schlitter *et al*. 1999). Despite the lack of chromosomal data for many species of *Crocidura*, karyotypic information has been used in a number of analyses of evolution and systematics of this taxonomically and karyotypically diverse genus (Maddalena and Ruedi 1994, Zima *et al*. 1998, Biltueva *et al*. 2001). Although some African rodents and bats have been well-studied, there are numerous species for which no karyotypic data are available.

Because some species are known to exhibit karyotypic variation within and among populations, it is important to examine karyotypes from multiple individuals from different localities. Much of the karyotypic work that has been done on small mammals in tropical Africa has focused on Ivory Coast, Cameroon, and Central African Republic. Some of Africa's most extensive tracts of tropical rainforest are found in Gabon, and the area supports a high level of biodiversity. Despite this diversity, relatively little work has been done on the karyology of small mammals in Gabon.

Under the auspices of the Smithsonian Institution, and with the support of Shell Foundation and Shell Gabon, we participated in a survey of the mammalian biodiversity of the Gamba Complex of Protected Areas. We sampled small mammals from the Rabi oil field, which is located in the rainforest just south of the equator in Gabon. Human activities at Rabi are restricted to relatively small insular clearings within extensive tracts of rainforest. As part of this survey, we prepared karyotypes from a number of species of small mammals, including shrews, rodents, and bats. We report here chromosomal data for the shrews, rodents, and the bats of the Suborder Megachiroptera. Standard karyotypes of microchi-

[1] Department of Biology, Xavier University of Louisiana, Drexel Drive, New Orleans, LA 70125, USA. Author for correspondence, Email: cporter@xula.edu

[2] Direction de la Faune et de la Chasse, BP 159, Tchibanga, Gabon. Email: guimondou@yahoo.fr

[3] Gabon Biodiversity Program, Smithsonian Institution, S/C Shell Gabon, BP 48, Gamba, Gabon. Email: mboumbamoussoundaditona@yahoo.fr

[4] IRAF - CENAREST, BP 2246, Libreville, Gabon. Email: nganguiraphael@yahoo.com

[5] Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, USA. Email: rjbaker@TTU.EDU

[6] Present address: School of Biological Sciences, University of Nebraska, Lincoln, NE 68588. USA. Email: federico@unlserve.unl.edu

ropteran bats will be reported in a separate publication. We compare the results with published data and discuss the systematic and evolutionary implications of the chromosomal variability in the taxa studied.

2 Materials and Methods

We collected small mammals from the Rabi oil field of the Gamba Complex in Ogooué-Maritime Province, Gabon (Lee *et al*. this volume; see map page xxxii). Animals were sampled during February and March of 2002 as described by Rodriguez *et al*. (this volume) and O'Brien *et al*. (this volume).

Mitotic chromosome preparations were performed in the laboratory at Rabi from bone marrow using the methods of Baker *et al*. (2003), with the exception that ethanol was used in the fixative due to the unavailability of methanol. Meiotic preparations were made for selected males using the same procedures with testicular tissue. Chromosomes were stained with giemsa. The Government of Gabon authorized the research and permitted retention of some specimens as vouchers.

Specimens were prepared as standard skin-and-skeleton or alcoholic preparations, and are deposited as vouchers in the mammal collections of the Gabon Biodiversity Program in Gamba, the Smithsonian Institution in Washington, or the Natural Science Research Laboratory at Texas Tech University. Chromosome slides and cell suspensions are archived with tissue samples at Texas Tech University, and are cross-referenced to the voucher specimens by a unique TK number. Specimens examined are listed in Table 1.

3 Results and Discussion

The karyotypes of 17 species (four species of insectivores, seven species of rodents, and six of bats) found in the Gamba Complex were studied, including karyotypes of five species not previously documented: *Crocidura crenata*, *C. goliath*, *C. grassei*, *Heimyscus fumosus*, and *Scotonycteris zenkeri*. For some other species, we describe karyotypes that differ in some respects from those previously reported in the literature. Our results document that the biodiversity at Rabi includes chromosomal as well as taxonomic aspects. Our field laboratory was not equipped for chromosomal banding procedures, so we have not yet applied chromosomal banding techniques to the specimens used in the study. Because of this, we cannot be certain of chromosomal homologies across species. Standard karyotype preparations frequently underestimate the extent of

Table 1. Specimens examined with observed diploid numbers (2n). TK = unique number for cross-reference to voucher specimen.

TK	Species	sex	2n	TK	Species	sex	2n
110269	*Crocidura crenata*	M	48?	110258	*Hypsignathus monstrosus*	F	36
110322	*Crocidura goliath*	M	50	110263	*Megaloglossus woermanni*	F	34
110417	*Crocidura goliath*	M	50	110267	*Megaloglossus woermanni*	M	34
110400	*Crocidura grassei*	M	40	110287	*Megaloglossus woermanni*	M	34
110262	*Sylvisorex ollula*	M	38	110290	*Megaloglossus woermanni*	F	34
110327	*Sylvisorex ollula*	M	38	110338	*Megaloglossus woermanni*	M	34
110285	*Heimyscus fumosus*	M	40	110264	*Myonycteris torquata*	F	36
110328	*Hylomyscus parvus*	F	46	110265	*Myonycteris torquata*	F	36
110368	*Hylomyscus stella*	M	46	110266	*Myonycteris torquata*	F	36
110389	*Hylomyscus stella*	M	46	110291	*Myonycteris torquata*	F	36
110415	*Lophuromys nudicaudus*	F	56	110292	*Myonycteris torquata*	F	36
110367	*Malacomys longipes*	M	48	110293	*Myonycteris torquata*	F	36
110397	*Mus musculoides*	F	34	110295	*Myonycteris torquata*	F	36
110398	*Mus musculoides*	M	34	110299	*Myonycteris torquata*	F	36
110302	*Praomys tullbergi*	F	34	110300	*Myonycteris torquata*	F	36
110251	*Epomops franqueti*	F	36	110339	*Myonycteris torquata*	F	36
110253	*Epomops franqueti*	F	36	110390	*Myonycteris torquata*	F	36
110254	*Epomops franqueti*	F	36	110392	*Myonycteris torquata*	M	36
110463	*Epomops franqueti*	F	36	110481	*Myonycteris torquata*	F	36
110475	*Epomops franqueti*	M	35	110465	*Scotonycteris zenkeri*	M	32

chromosomal rearrangements (Haiduk *et al*. 1981, Baker *et al*. 1987). Nevertheless, our results do provide insight into karyotypic evolution in some species, and also raise some questions that can be addressed by future banding studies.

3.1 Order Insectivora, Family Soricidae

Crocidura crenata

It is generally accepted that the genus *Crocidura* originated in Africa, and subsequently colonized Eurasia, probably during the late Miocene (Maddalena and Ruedi 1994, Butler 1998). The African and Eurasian lineages both underwent extensive speciation during the Pliocene and later. Maddalena and Ruedi (1994) proposed a 2n = 36-40 karyotype as primitive in the genus, with increasing diploid number in the African clade, and decreasing diploid number in the Eurasian branch of the genus. The majority of African *Crocidura* that have been studied have a 2n = 50 karyotype (Schlitter *et al*. 1999).

Crocidura crenata was described from Gabonese specimens in 1965, and has since been identified in Cameroon and the Democratic Republic of Congo (Hutterer and Schlitter 1996). Although the species has been considered rare, Goodman and Hutterer (2004) reported a series of 13 specimens from the Monts Doudou region of Gabon. The karyotype of this species has not been previously reported.

Chromosome preparations from a male of this shrew produced only a single spread of moderate quality (Fig. 1A). Based on this cell, the diploid number is 2n = 48. The chromosomes of this cell have some banding, although they were not treated with any banding agent. The banding was sufficient to help identify homologous chromosomes, but we were unable to clearly identify any homologies with G-banded chromosomes in other species of *Crocidura* (Biltueva *et al*. 1999, 2001). We were also unable to identify any sex chromosomes.

Because we examined only a single cell, we cannot exclude the possibility that the chromosome complement we observed is incomplete or otherwise not representative of the individual or species. A 2n = 48 karyotype is known from one other species of African *Crocidura* (*C. nigrofusca* from Burundi; Maddalena and Ruedi 1994, Schlitter *et al*. 1999).

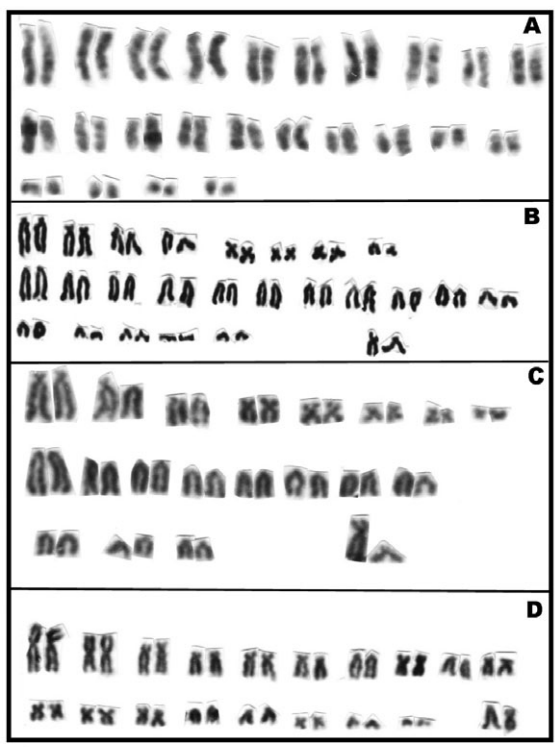

Figure 1. Standard karyotypes of shrews collected from the Rabi oil field. (A) *Crocidura crenata* male TK110269; (B) *Crocidura goliath* male TK110322; (C) *Crocidura grassei* male TK110400; (D) *Sylvisorex ollula* male TK110316.

Crocidura goliath

The taxonomic status of *C. goliath* has been unclear (see Nowak 1997). The taxon has been placed in a distinct genus or subgenus (*Praesorex*), and has at times been considered a subspecies of *C. odorata*. Molecular studies have placed *C. goliath* within a monophyletic *Crocidura* (Quérouil *et al*. 2001). Goodman *et al*. (2001) recorded the species in sympatry with *C. olivieri* in northeastern Gabon.

We karyotyped two males of *C. goliath* and found a diploid number of 50 in both individuals (Fig. 1B). The karyotype has three pairs of large subtelocentric autosomes and 17 pairs of acrocentric autosomes graded from large to small. Three additional small pairs are metacentric or submetacentric and one small pair is subtelocentric. The apparent sex chromosomes of *C. goliath* include a large submetacentric X and a large subtelocentric Y. The autosomes of *C. goliath* are identical to those in *C. odorata giffardi*, but the latter species differs from *C. goliath* in having a large metacentric X chromosome (Meylan and Vogel 1982). The X chromosome of *Crocidura* is generally a large metacentric, but some species are known to have a submetacentric X (Maddalena and Ruedi 1994).

Crocidura grassei

For 35 years after its description (Brosset *et al*. 1965), fewer than 10 specimens of *C. grassei* were reported from Gabon, Cameroon, Central African Republic, and Equatorial Guinea (Lasso *et al*. 1996, Goodman *et al*. 2001). However, recent studies (Goodman and Hutterer 2004, Nicolas et al. 2004) have shown the species to be locally abundant in some areas of Gabon. *Crocidura grassei* has not been previously karyotyped.

We karyotyped one male *C. grassei* from Rabi and found a diploid number of 40 (Fig. 1C). Five pairs of small autosomes are metacentric or submetacentric. Three autosomal pairs are subtelocentric, and ten are acrocentric. The remaining autosomal pair is heteromorphic with one chromosome being acrocentric and the other subtelocentric. The long arm of the subtelocentic chromosome is the same size as its acrocentric homologue. It is likely that the short arm is heterochromatic, and may be polymorphic in the population. G- and C-banding will be necessary to confirm that interpretation. The X chromosome is large and submetacentric. The Y is smaller and subtelocentric. Cells in the diakinesis stage of meiosis have homologous chromosomes paired to form the expected 20 bivalents (Fig. 2A-B).

The 2n = 40 karyotype of *C. grassei* is one of the lowest reported among African species, although a diploid number as low as 22 is known among Asian species of the genus (Zima *et al*. 1998). Meylan (1971) reported a similar 2n = 40 karyotype for the African species *C. bottegi*. The standard karyotypes of *C. grassei* and *C. bottegi* differ by (1) a possible pericentric inversion resulting in a medium-sized acrocentric in *C. grassei* and a metacentric in *C. bottegi*; (2) a longer short arm on the X chromosome in *C. grassei*; and (3) fixation in *C. bottegi* of the subtelocentric form of the heteromorphic pair of *C. grassei*.

There is a high level of karyotypic similarity between *C. grassei* and *C bottegi*, and with other African *Crocidura* with low (2n = 36-44) diploid numbers (Maddalena and Ruedi 1994, Schlitter *et al*. 1999). However, these karyotypes are very similar to the karyotype proposed as ancestral for the genus (Maddalena and Ruedi 1994, Zima *et al*. 1998), and do not necessarily indicate a close relationship among the African species that have low diploid numbers. Nonetheless, it is likely that these species diverged early in the diversification of African *Crocidura* and in some cases, that divergence may have predated the Eurasian radiation. Indeed, morphological, molecular and chromosomal studies have identified *C. bottegi* as a primitive taxon, which diverged prior to the split between the major African and Eurasian clades

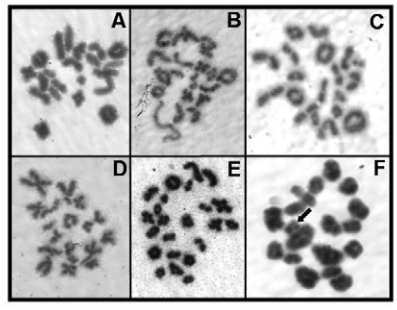

Figure 2. Meiotic chromosome preparations from small mammals collected at the Rabi oil field. All specimens illustrated are males. (A) Diakinesis from *Crocidura grassei* TK110400; (B-C) Diakinesis from *Sylvisorex ollula* TK110316; (D) Secondary spermatocyte from *Sylvisorex ollula* TK110316; (E) Diakinesis from *Hylomyscus stella* TK110389; (F) Diakinesis of *Epomops franqueti* TK110475. Arrow indicates possible univalent.

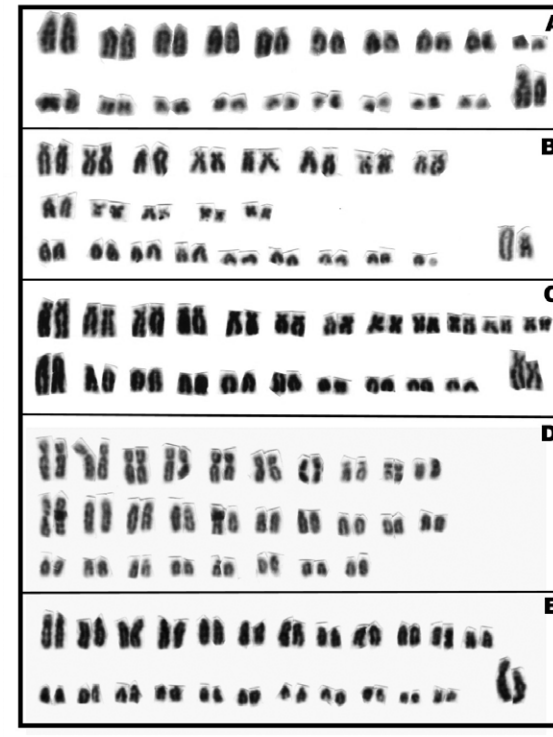

Figure 3. Standard karyotypes of rodents collected at the Rabi oil field. (A) *Heimyscus fumosus* male TK110285; (B) *Hylomyscus parvus* female TK110328; (C) *Hylomyscus stella* male TK110389; (D) *Lophuromys nudicaudus* female TK110415; (E) *Malacomys longipes* male TK110367.

(Maddalena and Ruedi 1994, Ruedi 1998). Based on morphology, Brosset *et al*. (1965) suggested a close relationship between *C. grassei* and *C. dolichura*, which has also been identified as one of the more primitive members of the genus (McClellan 1994, Butler 1998). The karyotype of *C. grassei* and its similarities with *C. bottegi* and *C. dolichura* suggest that it is derived from an early branch within the genus.

Sylvisorex ollula

Mitotic chromosome preparations from two males of *S. ollula* both yielded 2n = 38 (Fig. 1D). The karyotype has 13 pairs of biarmed chromosomes, and five pairs of acrocentric autosomes. The X chromosome is acrocentric and the Y is metacentric. We observed the expected 19 bivalents in diakinesis (Fig. 2C) and secondary spermatocytes have a haploid number of 19 (Fig. 2D). The autosomal complement is identical to that reported for two specimens collected in Cameroon by Schlitter *et al*. (1999). However, the Y chromosome is larger than was observed in the Cameroon specimens, and is metacentric rather than acrocentric. The Y we observed in the specimens from Rabi may differ in the amount of heterochromatin. This hypothesis can be tested by C-banding.

Schlitter *et al*. (1999) recognized two groups of *Sylvisorex* based on karyotype. The species *S. megalura* and *S. lunaris* have high diploid and fundamental numbers, whereas the species *S. johnstoni, S. isabellae, S. morio*, and *S. ollula* have karyotypes with low diploid and fundamental numbers. The systematic basis of this division was supported by analysis of 16S rRNA gene sequences (Quérouil *et al*. 2001), which suggests that *S. megalura* is more closely allied with *Suncus* than it is with the low-2n *Sylvisorex*, including *S. ollula*.

3.2 Order Rodentia, Family Muridae

Heimyscus fumosus

The karyotype of *Heimyscus* consists of 40 chromosomes (Fig. 3A). Three pairs of microchromosomes are biarmed and the remaining autosomes are acrocentric. The X is a large subtelocentric and the Y is a slightly smaller acrocentric. *H. fumosus* is known from Gabon, Cameroon, and Central African Republic. Brosset *et al*. (1965) described the species in the genus *Hylomyscus*. Subsequently, Misonne (1969) described *Heimyscus* as a new monotypic genus for this mouse, based on dental and cranial differences from *Hylomyscus*.

Robbins *et al*. (1980) did not describe a karyotype for *Heimyscus*, but reported that chromosomal differences were part of the justification for placing *H. fumosus* in a genus separate from *Hylomyscus*. Kingdon (1997) also referred to chromosomal characters that distinguish *Heimyscus* and *Hylomyscus*. Kingdon did not cite a reference, but it is likely that Robbins *et al*. (1980) may have been the source of Kingdon's information. Neither the genus description (Misonne 1969) nor the species description (Brosset *et al*. 1965) included any karyotypic information, and we have been unable to locate any published information on the karyotype of *Heimyscus*. According to D. Schlitter (pers. comm.), Robbins *et al*.'s (1980) report was based on a chromosomal preparation from *H. fumosus* that was not of sufficient quality to publish.

Although their conclusions were based on less than ideal data, it appears that Robbins *et al*. (1980) and Kingdon (1997) were correct in their assessment of karyotypic differences between *Heimyscus* and *Hylomyscus*. *Hylomyscus* species have a 2n = 46 karyotype with a substantial number of large biarmed chromosomes (Fig. 3B-C; Matthey 1963, Eisentraut 1969, Robbins *et al*. 1980, Viegas-Péquignot *et al*. 1983, Iskandar *et al*. 1988). Based on molecular data, Lecompte *et al*. (2002) confirmed that *H. fumosus* is not a member of *Hylomyscus*.

Based on morphological criteria, Misonne (1969) suggested a close relationship between *Heimyscus* and *Dephomys*, whereas Lecompte *et al*. (2002) suggested a close relationship with *Malacomys verschureni*. *Dephomys* was not included in Lecompte *et al*.'s (2002) molecular analysis. *Malacomys verschureni* was known from only a few specimens (Nowak 1997) and the species has been karyotyped (Robbins and Van der Straeten 1982). Two congeneric species, *M. edwardsi* and *M. longipes*, have a predominately acrocentric 2n = 48 karyotype (Fig. 3E; Matthey 1958, Viegas-Péquignot *et al*. 1983). The *Heimyscus* karyotype (Fig. 3A) differs from that of *Malacomys* (Fig. 3E) only by the addition of two acrocentric pairs, and by Robertsonian rearrangements that produced two small metacentrics. The morphology of the sex chromosomes is similar in the two genera. Although chromosomal data are consistent with a close relationship of *Heimyscus* and *Malacomys*, phylogenetic evaluation of cytochrome b affiliates *Heimyscus* with the Praomys group.

Tranier and Dosso (1979) reported karyotypes for *Dephomys eburnea* (2n = 42; 14 biarmed, 20 acrocentric) and *D. defua* (2n = 54; 48 biarmed, and 6 acrocentric). Based on the karyotypes, *Dephomys* seems less likely to be more closely related to *Heimyscus* than to *Malacomys*.

Hylomyscus parvus

A female of this species collected at Rabi has a diploid number of 46. The autosomes include 13 pairs of biarmed and 9 pairs of acrocentric chromosomes (Fig. 3B). The autosomal complement is identical to that described by Robbins *et al*. (1980) for *H. parvus* in Cameroon. However, our specimen from Gabon differs in the morphology of the sex chromosomes. Despite the fact that it is a female, our specimen appears to have heteromorphic X chromosomes with one X being large and acrocentric and the other being smaller and subtelocentric. We interpret this heteromorphic pair as sex chromosomes based on the fact that the remaining chromosomes are identical to the autosomes described by Robbins *et al*. (1980). Heteromorphic X chromosomes have been described in other species of rodents, and usually indicate autosomal translocations (Fig. 4A; Jotterand 1972, Zhu *et al*. 2003).

Hylomyscus stella

Some variation has been reported in the karyotypes of specimens of *H. stella*. Matthey (1963) reported two similar (2n = 46) karyotypes for individuals collected from the same locality in Congo. The "large" form had 13 pairs of biarmed and 9 pairs of acrocentric autosomes; the "small" form had 12 pairs of biarmed and 10 pairs of acrocentric autosomes. Matthey regarded the two forms as subspecies although he did not assign formal taxonomic names. Matthey's (1963) large form had a large submetacentric X and a small subtelocentric Y, while the small form had a more nearly metacentric X and an acrocentric Y.

Robbins *et al*. (1980) reported a female from Cameroon whose karyotype corresponds to Matthey's large form. Viegas-Péquignot *et al*. (1983) reported 2n = 46 karyotype from Central African Republic, with 12 pairs of biarmed and 10 pairs of acrocentric autosomes. Although the autosomes of Viegas-Péquignot *et al*.'s specimen resemble the autosomes of Matthey's small form, the sex chromosomes resemble those of the large form. Based on specimens from northeastern Gabon, Iskandar *et al*. (1988) reported a 2n = 46 karyotype with 11 biarmed and 11 acrocentric autosomal pairs. Iskandar *et al*. (1988) also described a sympatric 2n = 44 form which they recognized (but did not name) as a separate species, based on analysis of allozyme data.

We examined two males of *H. stella*, and both have a diploid number of 46 with 12 biarmed and 10 acrocentric pairs of autosomes (Fig. 3C). The autosomal complement, therefore, corresponds to Matthey's (1963) small form. The X and Y are both submetacentric, thus the X chromosome corresponds to that of Matthey's large form. The Y is distinctly submetacentric and has a longer short arm than Matthey illustrated for the Y chromosome of his large variety. Meiotic preparations produced diakinesis cells with 23 bivalents (Fig. 2E).

Based on the karyotypes that have been reported in the literature, it is clear that *H. stella* has chromosomal variability through much of its range in tropical Africa. Future studies of *H. stella* should use G-banding to document the karyotypic changes, and to document the extent of within- and between-population variation.

Lophuromys nudicaudus

A female of this species has a diploid number of 56, with ten pairs of metacentric and submetacentric chromosomes (Fig. 3D). The remaining 18 pairs are acrocentric. We were not able to identify sex chromosomes. The diploid number in *Lophuromys* ranges from 42 to 70 (Robbins and Baker 1978, Aniskin *et al*. 1997). Verheyen and Van der Straeten (1980) karyotyped a male and female of *L. nudicaudus* from Cameroon, and also reported a diploid number of 56 in both individuals. The cells they examined had 14 to 17 metacentric and submetacentric chromosomes. Verheyen and Van der Straeten (1980) observed that one or two acrocentric chromosomes had a secondary constriction. We did not see secondary constrictions in the cells we examined.

Malacomys longipes

Viegas-Péquignot *et al*. (1983) examined *M. longipes* from Ivory Coast and reported a 2n = 48 karyotype with 22 pairs of acrocentric and one pair of small metacentric autosomes. The X and Y chromosomes were both submetacentric, with the short arm of the Y being heterochromatic. Our standard karyotype of a single male from Gabon (Fig. 3E) was indistinguishable from the karyotype they reported.

Mus musculoides

The two animals we karyotyped of this species were part of a larger series of specimens collected from open areas associated with human habitations at Rabi. A male and female both had a diploid number of 34, and had identical autosomes (Fig. 4A-B). All autosomes are acrocentric. The largest chromosome in the karyotype is the X, which is submetacentric. The Y is also quite large and is subtelocentric. The female has heteromorphic X chromosomes (Fig. 4A). We interpret the smaller chromosome as a deleted X (X_d) which has been reported in other individuals of this complex (Jotterand-Bellomo 1984, 1986; Castiglia *et al*. 2002).

A considerable amount of karyotypic variability has been documented in this group, and the taxonomy is unsettled (Jotterand 1972, Castiglia *et al*. 2002). There is no consensus on the number of biological species represented in the group, and the name "*Mus minutoides/musculoides*" has been applied to these mice. The diploid number in the complex varies from 2n = 18 to 2n = 34 (Jotterand 1972, Castiglia *et al*. 2002).

G-banding studies have documented that this variation is the result of Robertsonian fusions, and that the sex chromosomes have been translocated onto autosomal pair 1 in specimens collected from Ivory Coast and Zambia (Jotterand-Bellomo 1984, 1986; Castiglia *et al*. 2002). Without G-banding, we cannot confirm this translocation in our specimens from Gabon, but the large submetacentric X seen in both the male and female suggests that the same translocation is involved. The autosomes are all acrocentric in our specimens from Rabi. The 2n = 34 karyotype is characteristic of the presumed ancestral karyotype (Jotterand-Bellomo 1984, 1986), differing only in the translocation of pair 1 to the sex chromosomes. Other karyotypes in the *Mus minutoides/musculoides* complex reflect a variable number of centric fusions.

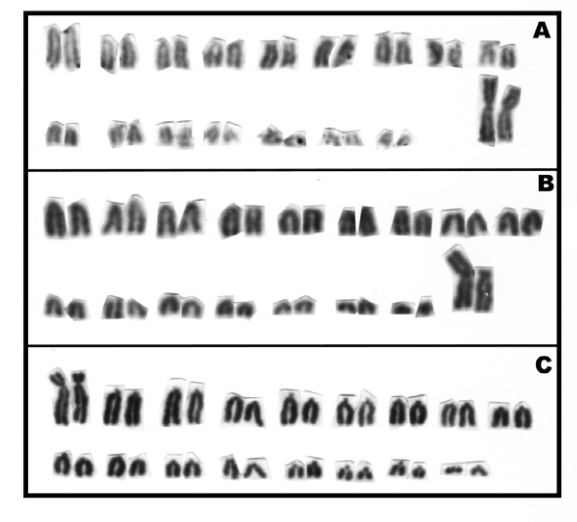

Figure 4. Standard karyotypes of rodents collected at the Rabi oil field. (A) *Mus musculoides* female TK110397; (B) *Mus musculoides* male TK110398; (C) *Praomys tullbergi* female TK110302.

Praomys tullbergi

A female *Praomys tullbergi* has a diploid number of 34 (Fig. 4C). The largest pair is subtelocentric and is slightly heteromorphic in the length of the short arm. We classify the remaining chromosomes as acrocentric, although very tiny short arms can be seen on a number of these chromosomes. The same karyotype was described by Matthey (1958) and by Petter (1975) for specimens from Ivory Coast and Central African Republic. Those authors described acrocentric X and Y chromosomes, but we were unable to identify which acrocentrics might be the X chromosomes in our female. Qumsiyeh *et al*. (1990) described a specimen from Kenya with 2n = 35, but that specimen probably represents an undescribed species.

3.3 Order Chiroptera, Family Pteropodidae

Epomops franqueti

Peterson and Nagorsen (1975) and Haiduk *et al*. (1980) both described a 2n = 36 karyotype from specimens of *E. franqueti* collected in Cameroon and Kenya. The karyotype from Kenya was described based on examination of two females, but the Cameroon sample included 4 females and one male. Haiduk *et al*. (1980) described a standard XX/XY sex chromosome system, with both sex chromosomes being subtelocentric. The X was of intermediate size and the Y was the smallest chromosome in the complement.

We karyotyped a male of this species and found a 2n = 35 karyotype with an autosomal complement identical to that described previously (Fig. 5 A-B). However, none of the cells examined had a Y chromosome. Diakinesis arrays have 18 elements, one of which appears to be an X chromosome univalent (Fig. 2F).

An XX / XO sex chromosome system is known in two species of *Epomophorus* (Peterson and Nagorsen 1975). It appears that there is some variation in the karyotype of male *Epomops*, but the origin of this variation is unknown. It is possible that at least some populations are polymorphic for the loss of the Y chromosome, or that there is geographic variation in sex chromosomes.

Hypsignathus monstrosus

Haiduk *et al*. (1980, 1981) examined females from Zaire and Cameroon and found a 2n = 36 karyotype with all chromosomes being biarmed. We karyotyped one female from Rabi and found an identical karyotype (Fig. 5C).

Megaloglossus woermanni

We karyotyped two females and three males of *M. woermanni*. All specimens had a 2n = 34 karyotype (Fig. 5D). All autosomes are biarmed with the exception of the smallest pair which is acrocentric. The sex chromosomes are both biarmed. The standard karyotype is identical to that found by Haiduk *et al*. (1980) in two specimens from Cameroon. The standard karyotype is similar to that seen in other pteropodid bats, although it has a smaller diploid number. Haiduk *et al*. (1981) used chromosomal banding techniques to determine chromosome arm homologies. They concluded that the karyotype of *Megaloglossus* has undergone two pericentric inversions, two translocations, and the addition of heterochromatin to the short arm of the X.

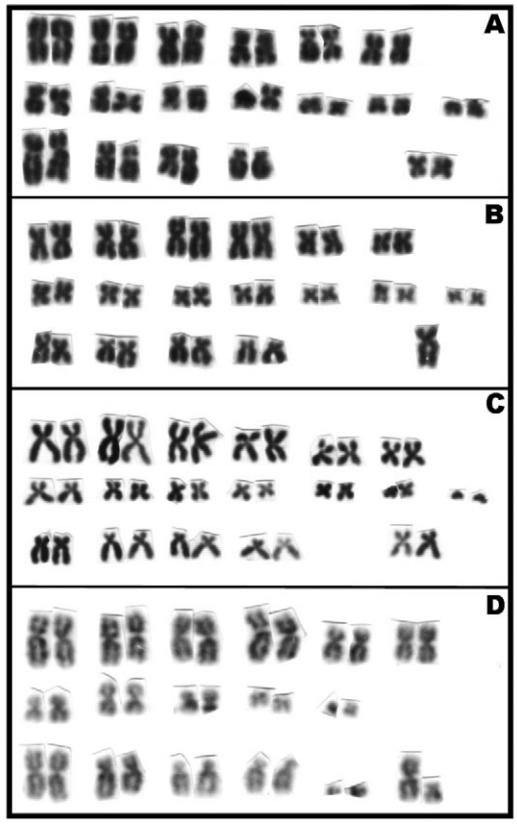

Figure 5. Standard karyotypes of megachiropteran bats collected at the Rabi oil field. (A) *Epomops franqueti* female TK110252; (B) *Epomops franqueti* male TK110475; (C) *Hypsignathus monstrosus* female TK110258; (D) *Megaloglossus woermanni* male TK110338.

Myonycteris torquata

We examined 12 females and one male of this species; all had a diploid number of 36 (Fig. 6A-B). All autosomes are biarmed with the exception of one pair of small acrocentrics. The X chromosome is subtelocentric and the Y is a very small acrocentric. The series included specimens of three different morphological variants. The karyotypes of the three forms were indistinguishable, and molecular evidence (Rodriguez *et al*. this volume) indicates that the morphological variants represent

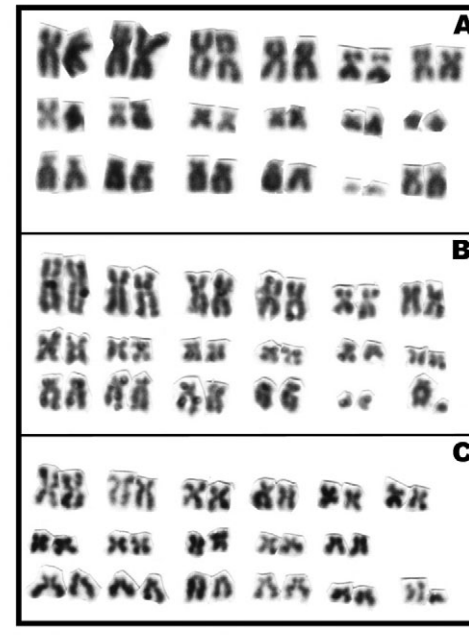

Figure 6. Standard karyotypes of megachiropteran bats collected at the Rabi oil field. (A) *Myonycteris torquata* female TK110264; (B) *Myonycteris torquata* male TK110392; (C) *Scotonycteris zenkeri* male TK110465.

a single population. Rodriguez *et al*. (this volume) interpreted the morphological variation as being a function of age.

Haiduk *et al*. (1980, 1981) found an identical karyotype in a specimen from Cameroon. Their specimen is heterozygous for a pericentric inversion in pair 1. The inversion was demonstrated with G-banding (Haiduk *et al*. 1981), but is also visible in the proportions of the long and short arms in the standard karyotype (Haiduk *et al*. 1980). We did not see evidence of this heteromorphism in the standard karyotypes of our specimens.

Scotonycteris zenkeri

One male of this species was karyotyped. The diploid number is 32 (Fig. 6C). With the exception of one pair of acrocentrics, all autosomes are distinctly biarmed. Ten pairs of autosomes are metacentric or submetacentric, and four are subtelocentric. The X chromosome is submetacentric. The Y chromosome is very small, but does not appear to be biarmed.

The genus *Scotonycteris* includes two species, *S. ophiodon* and *S. zenkeri*. Haiduk *et al*. (1980) described the standard karyotype of *S. ophiodon*. Based on non-differentially stained chromosomes, the 2n = 34 karyotype of *S. ophiodon* is similar to that of *M. torquata*, but is distinctive in that it has a larger subtelocentric pair, and it lacks the secondary constriction seen in a medium-sized metacentric pair in *M. torquata*. However, subsequent studies involving G- and C-banding (Haiduk *et al*. 1981) revealed that the karyotype of *S. ophiodon* has been radically reorganized in comparison with other pteropodid bats, and that standard chromosome preparations underestimate the amount of chromosomal change. Haiduk *et al*. (1981) hypothesized that the derivation of the *Scotonycteris* karyotype required a minimum of 13 chromosomal rearrangements in addition to five heterochromatic additions and a pericentric inversion in the X chromosome.

Our standard preparations of *S. zenkeri* revealed a 2n = 32 karyotype similar to that seen in *S. ophiodon*, but with some noteworthy differences. Both species of *Scotonycteris* have a single pair of acrocentric autosomes. However, in *S. ophiodon*, the smallest autosomal pair is acrocentric, whereas we found that in *S. zenkeri*, the smallest pair is subtelocentric, and one medium-sized pair is acrocentric. The chromosomes in our preparations are more contracted than those illustrated by Haiduk *et al*. (1980), so it is difficult to compare sizes, but *S. zenkeri* does not have the very large subtelocentric pair reported in *S. ophiodon*. Unlike *S. ophiodon*, the three largest subtelocentric pairs of *S. zenkeri* are all roughly the same size. Finally, the Y chromosome of *S. zenkeri* does not appear to be biarmed.

Both standard and banded chromosome preparations revealed that *S. ophiodon* is distinct from related fruit bats (Haiduk *et al*. 1980). Although the two species of *Scotonycteris* have similar karyotypes, we are cautious in assuming any chromosomal homologies without examining banded karyotypes.

References

Aniskin, V.M., L.A. Lavrenchenko, A.A. Varshaviskii and A.N. Milishikov. 1997. Karyotypic differentiation of three harsh-furred mouse species of Genus *Lophuromys* (Murinae, Rodentia) from the Bale Mountains National Park, Ethiopia. *Genetika* 33: 967-973.

Baker, R.J., M. Hamilton and D.A. Parish. 2003. Preparations of mammalian karyotypes under field conditions. *Occasional Papers, Museum of Texas Tech University* 228: 1-8.

Baker, R.J., M.B. Qumsiyeh and C.S. Hood. 1987. Role of chromosomal banding patterns in understanding mammalian evolution. *In*: Genoways, H.H., ed., *Current Mammalogy*, Vol.1. Plenum Publishing Corp., NY.

Biltueva, L.S., P.L. Perelman, M.B. Rogatcheva, E.L. Fedorova, N.V. Vorobieva, M. Harada, K. Mekada, S.I. Oda and A.S. Graphodatsky. 1999. Comparative cytogenetics of some species of *Crocidura* (Insectivora) with 2n=40. *Cytologia* 64: 293-299.

Biltueva, L.S., M.B. Rogatcheva, P.L. Perelman, P.M. Borodin, S.I. Oda, K. Koyasu, M. Harada, J. Zima and A.S. Graphodatsky. 2001. Chromosomal phylogeny of certain shrews of the genera *Crocidura* and *Suncus* (Insectivora). *Journal of Systematics and Evolutionary Research* 39: 69-76.

Brosset, A., G. Dubost and H. Heim de Balsac. 1965. Mammifères inédits récoltés au Gabon. *Biologia Gabonica* 1: 147-174.

Butler, P.M. 1998. Fossil history of shrews in Africa. *In*: Wójcik, J.M. and M. Wolsan, eds., *Evolution of Shrews*. Polish Academy of Sciences, Bialowieza, Poland.

Castiglia, R., E. Gornung and M. Corti. 2002. Cytogenetic analyses of chromosomal rearrangements in *Mus minutoides/musculoides* from northwest Zambia through mapping of the telomeric sequence $(TTAGGG)_n$ and banding techniques. *Chromosome Research* 10: 399-406.

Eisentraut, M. 1969. Die Verbreitung der Muriden-Gattung *Hylomyscus* auf Fernando Poo und in Westkamerun. *Zeitschrift für Säugetierkunde* 34: 296-307.

Goodman, S.M. and R. Hutterer. 2004. A report on the shrews (Mammalia: Soricidae) of Monts Doudou, Gabon: elevational distribution and preliminary insights into their ecology. Pp. 93-105 *in*: Fisher, B.L., ed., *Monts Doudou, Gabon, a floral and faunal inventory with reference to elevational variation.* No. 28, Memoirs of the California Academy of Sciences. San Francisco, CA.

Goodman, S.M., R. Hutterer and P.R. Ngnegueu. 2001. A report on the community of shrews (Mammalia: Soricidae) occurring in the Minkébé Forest, northeast Gabon. *Mammalian Biology* 66: 22-34.

Haiduk, M.W., R.J. Baker, L.W. Robbins and D.A. Schlitter. 1981. Chromosomal evolution in African Megachiroptera: G- and C-band assessment of the magnitude of change in similar standard karyotypes. *Cytogenetics and Cell Genetics* 29: 221-232.

Haiduk, M.W., L.W. Robbins, R.L. Robbins and D.A. Schlitter. 1980. Karyotypic studies of seven species of African megachiropterans (Mammalia: Pteropodidae). *Annals of Carnegie Museum of Natural History* 49: 181-191.

Hutterer, R. and D.A. Schlitter. 1996. Shrews of Korup National Park, Cameroon, with the description of a new *Sylvisorex* (Mammalia: Soricidae). *In*: Genoways, H.H. and R.R. Baker, eds., *Contributions in Mammalogy: A Memorial Volume Honoring Dr. J. Knox Jones, Jr.* Museum of Texas Tech University, Lubbock, TX.

Iskandar, D., J.M. Duplantier, F. Bonhomme, F. Petter and L. Thaler. 1988. Mise en évidence de deux espèces jumelles sympatriques du genre *Hylomyscus* dans le nord-est du Gabon. *Mammalia* 52: 126-129.

Jotterand, M. 1972. Le polymorphisme chromosomique des *Mus* (Leggadas) africains: cytogénétique, zoogéographie, évolution. *Revue Suisse de Zoologie* 79: 287-359.

Jotterand-Bellomo, M. 1984. L'analyse cytogénétique de deux espèces de Muridae africains, *Mus oubanguii* et *Mus minutoides*/*musculoides*: Polymorphisme chromosomique et ébauche d'une phylogénie. *Cytogenetics and Cell Genetics* 38: 182-188.

Jotterand-Bellomo, M. 1986. Le genre *Mus* africain, un exemple d'homogénéité caryotypique: étude cytogénétique de *Mus minutoides/musculoides* (Côte d'Ivoire), de *M. setulosus* (République Centrafricaine), et de *M. mattheyi* (Burkina Faso). *Cytogenetics and Cell Genetics* 42: 99-104.

Kingdon, J. 1997. *The Kingdon Field Guide to African Mammals*. Academic Press, San Diego, CA.

Lasso, C., R. Hutterer and A. Rial. 1996. Records of shrews (Soricidae) from Equatorial Guinea, especially from Monte Alen National Park. *Mammalia* 60: 69-76.

Lecompte, E., L. Granjon, J. Kerbis Peterhans and C. Denys. 2002. Cytochrome b-based phylogeny of the *Praomys* group (Rodentia, Murinae): a new African radiation? *Comptes Rendus Biologies* 325: 827-840.

Lee, M.E., A. Alonso, P. Campbell, F. Dallmeier and O.S.G. Pauwels. 2006. The Gamba Complex of Protected Areas: an illustration of Gabon's biodiversity. *In*: Alonso, A., M.E. Lee, P. Campbell, O.S.G. Pauwels and F. Dallmeier, eds., *Gamba, Gabon: Biodiversity of an Equatorial African Rainforest*. Bulletin of the Biological Society of Washington, No. 12.

Maddalena, T. and M. Ruedi. 1994. Chromosomal evolution in the genus *Crocidura* (Insectivora: Soricidae). Pp. 335-344 *in*: Merritt, J.F., G.L. Kirkland, Jr. and R.K. Rose, eds., *Advances in the Biology of Shrews*. Special Publications of Carnegie Museum of Natural History 18.

Matthey, R. 1958. Les chromosomes et la position systématique de quelques Murinae africains (Mammalia - Rodentia). *Acta Tropica* 15: 97-117.

Matthey, R. 1963. La formule chromosomique chez sept espèces et sous-espèces de *Murinae* africains. *Mammalia* 27: 157-176.

McLellan, L.J. 1994. Evolution and phylogenetic affinities of the African species of *Crocidura*, *Suncus*, and *Sylvisorex* (Insectivora: Soricidae). Pp. 379-391 *in*: Merritt, J.F., G.L. Kirkland, Jr. and R.K. Rose, eds., *Advances in the Biology of Shrews*. Special Publications of Carnegie Museum of Natural History 18.

Meylan, A. 1971. Chromosomes de Soricidés de Côte d'Ivoire (Mammalia, Insectivora). *Revue Suisse de Zoologie* 78: 603-613.

Meylan, A. and P. Vogel. 1982. Contribution à la cytotaxonimie de Soricidés (Mammalia, Insectivora) de l'Afrique occidentale. *Cytogenetics and Cell Genetics* 34: 83-92.

Misonne, X. 1969. African and Indo-Australian Muridae: Evolutionary Trends. *Annales du Musée Royal de l'Afrique Centrale* (Tervuren, Belgium) ser. IN-8, 172: 1-219.

Nicolas, V., P. Barrière, S. Guimondou and M. Colyn. 2004. Variabilité structurale des peuplements forestiers de rongeurs (Muridae) et musaraignes (Soricidae) dans les Monts Doudou, Gabon. Pp. 107-127 *in*: Fisher, B.L., ed., *Monts Doudou, Gabon, a floral and faunal inventory with reference to elevational variation.* No. 28, Memoirs of the California Academy of Sciences. San Francisco, CA.

Nowak, R.M. 1997. *Walker's Mammals of the World*. Johns Hopkins University Press, Baltimore, MD.

O'Brien, C., W. McShea, S. Guimondou, P. Barrière and M. Carleton. 2006. Terrestrial small mammals (Soricidae and Muridae) from the Gamba Complex of Protected Areas in Gabon: species composition and comparison of sampling techniques. *In*: Alonso, A., M.E. Lee, P. Campbell, O.S.G. Pauwels and F. Dallmeier, eds., *Gamba, Gabon: Biodiversity of an Equatorial African Rainforest*. Bulletin of the Biological Society of Washington, No. 12.

Peterson, R.L. and D.W. Nagorsen. 1975. Chromosomes of fifteen species of bats (Chiroptera) from Kenya and Rhodesia. *Life Sciences Occasional Papers, Royal Ontario Museum* 27: 1-14.

Petter, F. 1975. Les *Praomys* de République Centrafricaine (Rongeurs, Muridés). *Mammalia* 39: 51-56.

Quérouil, S., R. Hutterer, P. Barrière, M. Colyn, J.C. Kerbis Peterhans and E. Verheyen. 2001. Phylogeny and evolution of African shrews (Mammalia: Soricidae) inferred from 16s rRNA sequences. *Molecular Phylogenetics and Evolution* 20: 185-195.

Qumsiyeh, M.B., S.W. King, J. Arroyo-Cabrales, I.R. Aggundey, D.A. Schlitter, R.J. Baker and K.J. Morrow, Jr. 1990. Chromosomal and protein evolution in morphologically similar species of *Praomys sensu lato* (Rodentia, Muridae). *Journal of Heredity* 81: 58-65.

Reumer, J.W.F. and A. Meylan. 1986. New developments in vertebrate cytotaxonomy. IX. Chromosome numbers in the order Insectivora (Mammalia). *Genetica* 70: 119-151.

Ruedi, M. 1998. Protein evolution in shrews. *In*: Wójcik, J.M. and M. Wolsan, eds., *Evolution of Shrews*. Polish Academy of Sciences, Bialowieza, Poland.

Robbins, L.W. and R.J. Baker. 1978. Karyotypic data for African mammals, with a description of an in vivo bone marrow technique. *Bulletin of Carnegie Museum of Natural History* 6: 188-210.

Robbins, C.B. and E. Van der Straeten. 1982. A New specimen of *Malacomys verschureni* from Zaire, Central Africa (Rodentia, Muridae). Revue de Zoologie Africaine, 96:216-220

Robbins, L.W., J.R. Choate and R.L. Robbins. 1980. Nongeographic and interspecific variation in four species of *Hylomyscus* (Rodentia: Muridae) in southern Cameroon. *Annals of Carnegie Museum* 49: 31-49.

Rodriguez, R.M., F.G. Hoffmann, C.A. Porter and R.J. Baker. 2006. The bat community of the Rabi Oilfield, in the Gamba Complex, Gabon. *In*: Alonso, A., M.E. Lee, P. Campbell, O.S.G. Pauwels and F. Dallmeier, eds., *Gamba, Gabon: Biodiversity of an Equatorial African Rainforest*. Bulletin of the Biological Society of Washington, No. 12.

Schlitter, D.A., R. Hutterer, T. Maddalena and L.W. Robbins. 1999. New karyotypes of shrews (Mammalia: Soricidae) from Cameroon and Somalia. *Annals of Carnegie Museum* 68: 1-14.

Tranier, M. and H. Dosso. 1979. Recherches caryotypiques sur les rongeurs de Côte d'Ivoire: Résultats préliminaires pour les milieux fermés. *Mammalia* 43: 254-256.

Verheyen, W.N. and E. Van der Straeten. 1980. The caryotype of *Lophuromys nudicaudus* Heller 1911 (Mammalia – Muridae). *Revue de Zoologie Africaine* 94: 311-316.

Viegas-Péquignot, E., B. Dutrilaux, M. Prod'Homme and F. Petter. 1983. Chromosomal phylogeny of Muridae: a study of 10 genera. *Cytogenetics and Cell Genetics* 35: 269-278.

Zhu, B., H. Gao, H. Wang, J. Gao, Y. Zhang, Y. Dong, J. Hou and X. Nan. 2003. The origin of the genetical diversity of *Microtus mandarinus* chromosomes. *Hereditas* 139: 90-95.

Zima, J., L. Lukácová and M. Macholán. 1998. Chromosomal evolution in shrews. *In*: Wójcik, J.M. and M. Wolsan, eds. *Evolution of Shrews*. Polish Academy of Sciences, Bialowieza, Poland.