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Chromosomal evolution in tenrecs (*Microgale* and *Oryzorictes*, Tenrecidae) from the Central Highlands of Madagascar

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Abstract

Tenrecs (Tenrecidae) are a widely diversified assemblage of small eutherian mammals that occur in Madagascar and Western and Central Africa. With the exception of a few early karyotypic descriptions based on conventional staining, nothing is known about the chromosomal evolution of this family. We present a detailed analysis of G-banded and molecularly defined chromosomes based on fluorescence *in situ* hybridization (FISH) that allows a comprehensive comparison between the karyotypes of 11 species of two closely related Malagasy genera, *Microgale* (10 species) and *Oryzorictes* (one species), of the subfamily Oryzorictinae. The karyotypes of *Microgale taiva* and *M. parvula* (2n = 32) were found to be identical to that of *O. hova* (2n = 32) most likely reflecting the ancestral karyotypes of both genera, as well as that of the Oryzorictinae. Parsimony analysis of chromosomal rearrangements that could have arisen following Whole Arm Reciprocal Translocations (WARTs) showed, however, that these are more likely to be the result of Robertsonian translocations. A single most parsimonious tree was obtained that provides strong support for three species associations within *Microgale*, all of which are consistent with previous molecular and morphological investigations. By expanding on a recently published molecular clock for the Tenrecidae we were able to place our findings in a temporal framework that shows strong chromosomal rate heterogeneity within the Oryzorictinae. We use these data to critically examine the possible role of chromosomal rearrangements in speciation within *Microgale*.

Introduction

One of the intriguing events in the evolutionary history of Afrotheria is the colonization of Madagascar by the afrosoricid family, Tenrecidae. After crossing the Mozambique Channel (probably by rafting from East Africa between 42 and 25 million years ago; Poux *et al.* 2005), the ancestors of the Malagasy tenrecs gave rise to an assemblage comprising eight genera that is virtually unique among eutherians in its

morphological diversity, and range of ecological adaptations (Olson & Goodman 2003). Recent phylogenies based on molecular and morphological characters strongly support two main clades within Malagasy tenrecs. The first corresponds to the subfamily Tenrecinae and includes four genera of spiny, hedgehog-like tenrecs (*Echinops*, *Hemicentetes*, *Tenrec* and *Setifer*). The second clade groups the semi-aquatic *Limnogale*, the shrew-like *Microgale*, and the mole-like *Oryzorictes* in the subfamily Oryzorictinae (Poux *et al*. 2005, Asher & Hofreiter 2006). The placement of the mouse-like *Geogale* is, however, uncertain, being either sister to all Malagasy tenrecs as a separate subfamily, the Geogalinae (Olson & Goodman 2003), or occupying an unresolved position within Oryzorictinae (Asher & Hofreiter 2006). Within Oryzorictinae, Olson & Goodman (2003) found that *Limnogale* nested within *Microgale*, a position that was supported by several non-ambiguous molecular characters. Moreover, although subsequent studies included only one species of *Microgale*, and could therefore not test the monophyly of this genus, strong support was found for an (*Oryzorictes*, (*Limnogale* + *Microgale*)) topology by Poux *et al*. (2005) and Asher & Hofreiter (2006).

With its 34 species (including three African species), the Tenrecidae is the most diversified family within Afrotheria, an endemic African clade of mammals that includes the Paenungulata (elephants, sirenids and hyraxes), and the Afroinsectiphillia comprising elephant shrews, tenrecs, golden moles and the aardvark (see Springer *et al*. 1997, Murphy *et al*. 2001, reviewed in Robinson & Seiffert 2004). This high diversity within the Tenrecidae can be attributed to the spectacular radiation of *Microgale*, the most speciose terrestrial mammal genus on Madagascar (Goodman *et al*. 2006). Most of these small, shrew-like tenrecs are found in the eastern humid forests of the island where they generally have a broad distribution, with many species occurring sympatrically (Jenkins 2003). The taxonomy of the genus has undergone extensive revision since its original description by Thomas (1882). For example, MacPhee (1987) retains only 10 of the 22 species described during the preceding century. During the past 20 years, however, extensive field surveys coupled with comprehensive morphometric and/or molecular investigations resulted in a considerable refinement of their taxonomy and patterns of distribution, with 11 new or resurrected species recognized in newer treatments (e.g., Jenkins 1993, Jenkins *et al*. 1997, Jenkins & Goodman 1999, Goodman & Soarimalala 2004, Olson *et al*. 2004, Goodman *et al*. 2006). Several other aspects of the biology of *Microgale* have received attention in the relatively recent past including physiology and reproduction (Stephenson & Racey, 1995 and references therein), as well as the morphology of its hind limb musculature (Endo *et al*. 2006), cranium (Asher 2001), placenta (Enders *et al*. 2006), male reproductive tract (Bedford *et al*. 2004) and spermatozoa (Bedford 2004). However, apart from karyotypic data from three species based on standard stained preparations (Borgaonkar & Gould 1968, 1969), virtually nothing is known about the chromosomal evolution of *Microgale*. This observation can in fact extend to other Tenrecidae since the only information available on the karyotypes of the other genera is restricted to diploid chromosome numbers and/or the occasional presentation of unbanded karyotypes (i.e., *Micropotamogale*, 2n = 40; *Tenrec* and *Hemicentetes*, 2n = 38; *Echinops* and *Setifer*, 2n = 40; Borgaonkar 1967, Borgaonkar & Gould 1965, Bernischke 1969). Moreover, there is no well-supported phylogenetic hypothesis detailing interspecific relationships within *Microgale*. Olson & Goodman's (2003) cladistic analysis of the Tenrecidae is the most comprehensive in terms of taxonomic sampling, including a large number of species of shrew tenrecs. Importantly, however, relationships within *Microgale* were not discussed in this study. Finally, although two recent studies have utilized both molecular and morphometric characters to define species limits in selected shrew tenrec taxa (Olson *et al*. 2004, Goodman *et al*. 2006), neither was intended to produce a comprehensive phylogeny for the genus.

Here, we use conventional banding as well as chromosome painting by fluorescence *in-situ hybridization* (FISH) to describe and compare the karyotypes of 10 *Microgale* species and that of the closely related genus *Oryzorictes*, represented by *O. hova*. We interpret the observed rearrangements in a cladistic framework and examine the outcomes with respect to two hypotheses of chromosomal evolution—one involving Whole Arm Reciprocal Translocations (WARTs), and the other only Robertsonian translocations. We place our findings in a temporal framework by expanding the molecular clock analysis of Poux *et al*. (2005) and show that extreme rate

differences exist in the chromosomal evolution of *Microgale* spp. Using these data, we critically examine the role for chromosomal rearrangements in speciation within the genus.

Material and methods

Tissue samples, cell culture and chromosome preparation

Tissue samples were collected during three inventory surveys of study sites situated in the rainforests of the Central Highlands of Madagascar (Table 1). Fibroblast cell lines were established using DMEM or Amniomax (Gibco: Paisley, Scotland, UK) culture medium supplemented with 15% fetal calf serum at 37°C and 5% CO_2. Chromosome harvests and G-banding were performed following conventional procedures (Seabright 1971) except that the concentration of the trypsin was decreased to 0.0025% for G-banding. Chromosomes were ordered in decreasing size and centromere position or according to the *M. taiva* format when the chromosome complement was conserved.

Table 1. List of species included in this study and associated voucher numbers of the specimens

Species	Site no.	Voucher no.
Microgale cowani	3	FMNH 194138
M. dobsoni	3	FMNH 194140
M. fotsifotsy	2	FMNH 188723
M. longicaudata	3	FMNH 194143
M. majori	2	FMNH 188726
M. parvula	2	FMNH 188729
M. principula	3	FMNH 194146
M. soricoides	2	FMNH 188732
M. thomasi	2	FMNH 188744
M. taiva	1	FMNH 178756
Oryzorictes hova	3	FMNH 194150

Site 1: surveyed in November 2003, Province de Fianarantsoa, Parc National de Midongy-Sud, NE slope of Mt. Papango, 3.5 km SW Befotaka, 23°50.3′S, 46° 57.5′E, alt. 1250 m.
Site 2: surveyed in January 2006, Province d'Antananarivo, Fivondronana d'Anjozorobe, Forêt d'Iaban'Ikoto, 5.5 km E Alakamisy, 18°31.3′S, 47°58.4′E, alt. 1280 m.
Site 3: surveyed in January 2007, Province d'Antananarivo, Réserve Spéciale d'Ambohitantely, Jardin Botanique, 18°10.3′S, 47°16.9′E, alt. 1450 m.
The locations of Anjozorobe and Ambohitantely are illustrated in Olson *et al*. (2004). All specimens are housed in the Field Museum of Natural History (FMNH).

Fluorescence in-situ hybridization (FISH)

Chromosome specific painting probes were generated from flow-sorted suspensions of Hoechst 33258 and chromomycin A-3 stained chromosomes that were separated on size and AT:GC ratio, and labelled with DOP-PCR (Telenius *et al*. 1992). FISH followed the protocol described in Gilbert *et al*. (2006) with the exception that chromosome preparations were denatured for 10 s in 70% formamide/0.6% SSC solution at 65°C rather than 30–45 s at the temperature of 70°C originally described. Biotin-labelled probes were detected by Cy3-avidin. Slides were mounted in antifade (Vectashield: Burlingame, California, USA) and images were captured using Genus 3.7 software (Applied Imaging).

Parsimony analysis

Parsimony analyses of the chromosomal rearrangements characterizing the interspecific relationships within *Microgale* were conducted by scoring chromosomal changes as characters and their presence/absence as the character states (Dobigny *et al*. 2004). Ancestral karyotypes for *Microgale*, *Oryzorictes* and the Oryzorictinae could be inferred *a priori* (see Results and Discussion). These ancestral karyotypes were used to polarize the characters. Consistent differences in G-banding patterns were observed in two instances that probably reflect complex intrachromosomal rearrangements. We could not assess the precise nature of these rearrangements but included them in the analyses since they result in distinct, easily identifiable G-banding patterns and therefore might be of interest to future studies that include other species of *Microgale*. These rearrangements were coded as 'presence/absence of an undetermined intrachromosomal change' (Table 2). Additionally, patterns corresponding to what would be anticipated following a Whole Arm Reciprocal Translocation (WART) were observed in some instances (Winking 1986, Searle *et al*. 1990). This type of rearrangement necessitates an exchange between chromosomal arms of two metacentrics, between one metacentric and one acrocentric, or between two metacentrics and one acrocentric chromosome (respectively type a, b and c WART in Hauffe & Pialek 1997). The effect of these rearrangements on fitness is thought to vary depending on the type of WART involved in the rearrangement, with

Table 2. Matrices of taxa/characters, (a) including whole-arm reciprocal translocations (WARTs), or (b) considering only fusions and fissions. Characters in bold are present in both matrices. Chromosomal changes are considered to be characters and their presence (1) / absence (0) the character states

Characters		OHO	MTA	MPA	MDO	MMA	MLO	MPR	MSO	MFO	MCO	MTH
(a)												
1	**Fi 2**	0	0	0	0	0	0	0	1	1	1	1
2	**Fi 3**	0	0	0	0	0	0	0	0	0	0	1
3	**Fi 4**	0	0	0	0	0	0	0	0	0	1	1
4	**Fi 5**	0	0	0	0	0	0	0	0	0	0	1
5	**Fi 6**	0	0	0	0	0	0	0	0	0	0	1
6	**Fi 7**	0	0	0	0	0	0	0	0	0	0	1
7	**Fi 8**	0	0	0	0	0	0	0	0	0	1	1
8	**Fi 9**	0	0	0	0	0	0	0	0	0	0	1
9	**Fi 10**	0	0	0	0	0	0	0	0	0	0	1
10	**Fi 10dist**	0	0	0	0	0	0	0	0	0	0	1
11	**Fi 11**	0	0	0	0	0	0	0	0	0	0	1
12	**Fi 13**	0	0	0	0	0	0	0	0	0	0	1
13	**Fu 12 + 14**	0	0	0	0	1	1	1	0	0	0	0
14	**Fu 9 + 14**	0	0	0	1	0	0	0	0	0	0	0
15	**Fu 7q + 14**	0	0	0	0	0	0	0	1	1	0	0
16	**undet. intra-chr. change 1**	0	0	0	0	0	0	1	0	0	0	0
17	**undet. intra-chr. change 2**	0	0	0	0	1	1	1	0	0	0	0
a	W(a) 4/5	0	0	0	0	0	0	0	1	1	0	0
b	W(a) 4/5	0	0	0	0	0	0	0	0	0	1	0
c	W(b) 7/12	0	0	0	0	0	0	0	1	1	0	0
d	W(a) 3/7	0	0	0	0	0	0	0	0	0	1	0
e	W(c) 6/11/9	0	0	0	0	0	0	0	0	0	1	0
(b)												
1	**Fi 2**	0	0	0	0	0	0	0	1	1	1	1
2	**Fi 3**	0	0	0	0	0	0	0	0	0	1	1
3	**Fi 4**	0	0	0	0	0	0	0	1	1	1	1
4	**Fi 5**	0	0	0	0	0	0	0	1	1	1	1
5	**Fi 6**	0	0	0	0	0	0	0	0	0	1	1
6	**Fi 7**	0	0	0	0	0	0	0	1	1	1	1
7	**Fi 8**	0	0	0	0	0	0	0	0	0	1	1
8	**Fi 9**	0	0	0	0	0	0	0	0	0	0	1
9	**Fi 10**	0	0	0	0	0	0	0	0	0	0	1
10	**Fi 10dist**	0	0	0	0	0	0	0	0	0	0	1
11	**Fi 11**	0	0	0	0	0	0	0	0	0	1	1
12	**Fi 13**	0	0	0	0	0	0	0	0	0	0	1
13	**Fu 12 + 14**	0	0	0	0	1	1	1	0	0	0	0
14	**Fu 9 + 14**	0	0	0	1	0	0	0	0	0	0	0
15	**Fu 7q + 14**	0	0	0	0	0	0	0	1	1	0	0
16	**undet. intra-chr. change 1**	0	0	0	0	0	0	1	0	0	0	0
17	**undet. intra-chr. change 2**	0	0	0	0	1	1	1	0	0	0	0
f	Fu 7p + 12	0	0	0	0	0	0	0	1	1	0	0
g	Fu 5q + 4q	0	0	0	0	0	0	0	1	1	0	0
h	Fu 5p + 4p	0	0	0	0	0	0	0	1	1	0	0
i	Fu 5q + 12	0	0	0	0	0	0	0	0	0	1	0
j	Fu 3p + 7q	0	0	0	0	0	0	0	0	0	1	0
k	Fu 3q + 7p	0	0	0	0	0	0	0	0	0	1	0
l	Fu 6p + 9	0	0	0	0	0	0	0	0	0	1	0
m	Fu 6q + 11p	0	0	0	0	0	0	0	0	0	1	0

Fi = fission; Fu = fusion; W(a) = WART between two metacentric chromosomes (type a WART in Hauffe & Pialek 1997); W(b) = WART between one metacentric and one acrocentric chromosome (type b WART in Hauffe & Pialek 1997); W(c) = WART between two metacentric chromosomes and one acrocentric chromosome (type c WART in Hauffe & Pialek 1997); undet. intra-chr. change = undetermined chromosomal change (see Material and methods for more details). Numbers associated with rearrangements refer to *Microgale taiva* chromosomes. See Figure 3 for species name abbreviations.

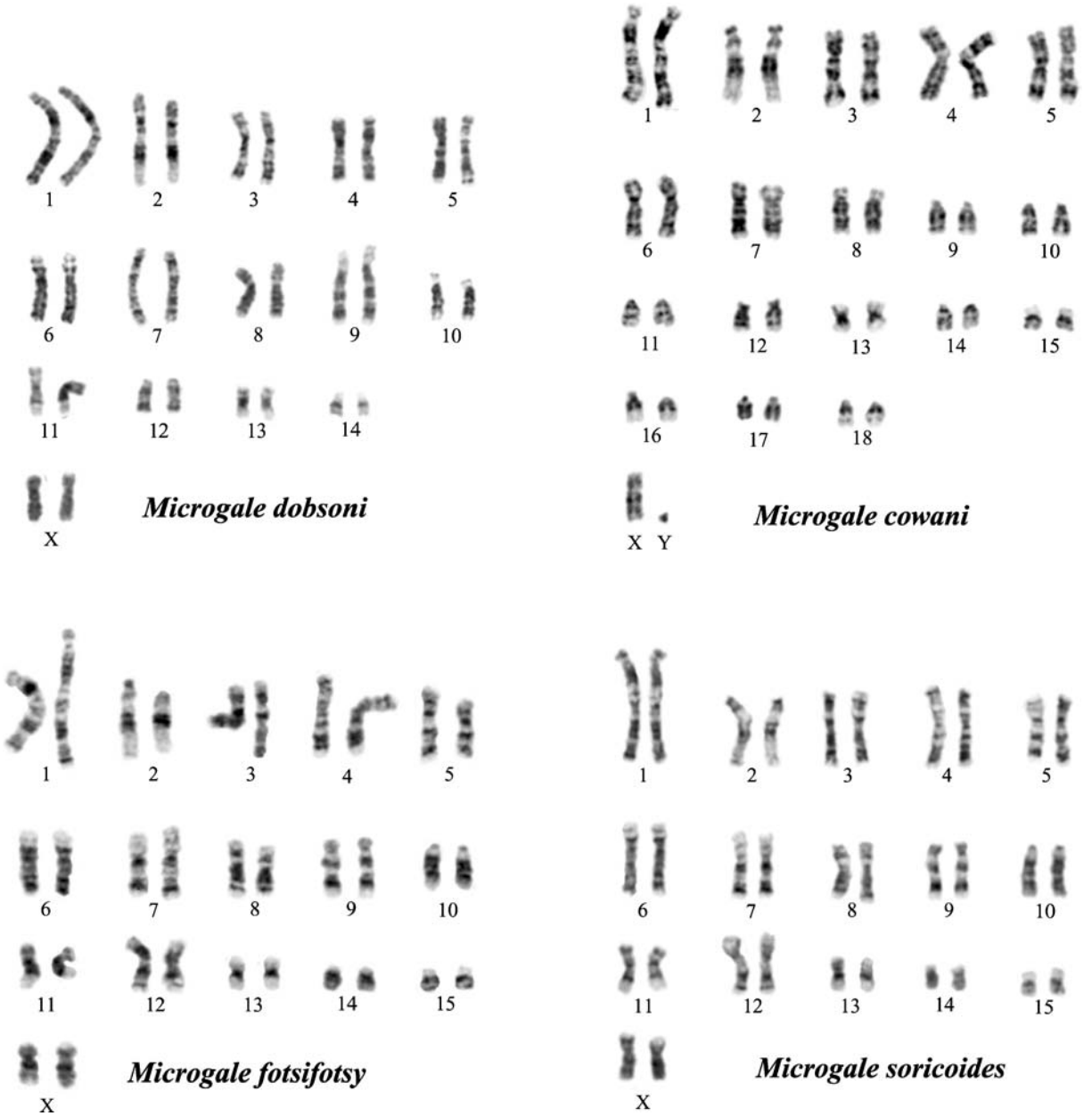
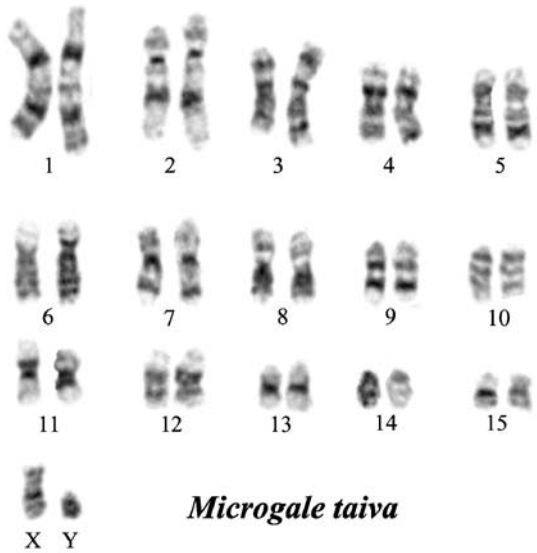
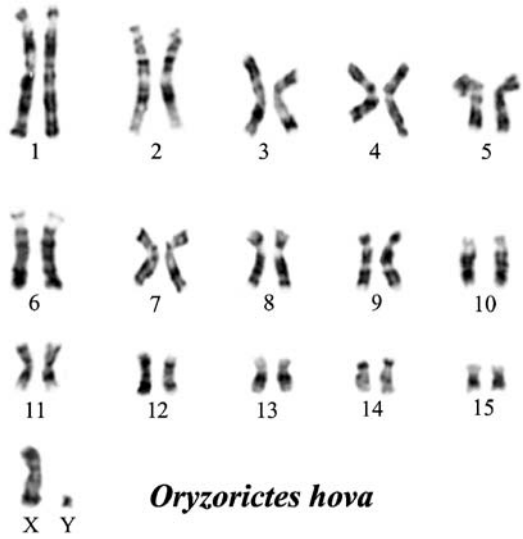
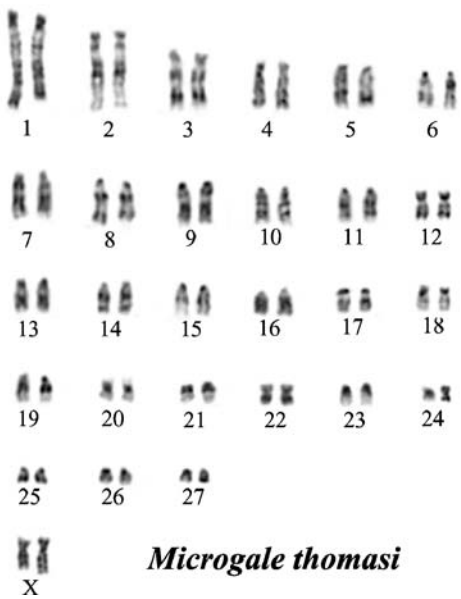


Figure 1. G-banded karyotypes of the 11 species of the Oryzorictinae included in this study: female *Microgale dobsoni* (2n = 30; FMNH 194140), male *M. cowani* (2n = 38; FMNH 194138), female *M. fotsifotsy* (2n = 32; FMNH 188723), female *M. soricoides* (2n = 32; FMNH 188732), male *M. taiva* (FMNH 178756), male *Oryzorictes hova* (FMNH 194150), female *M. thomasi* (FMNH 188744), male *M. parvula* (FMNH 188729), male *M. longicaudata* (FMNH 194143), female *M. principula* (FMNH 194146), female *M. majori* (FMNH 188726).

types c and b likely to be more detrimental than type a (Searle 1993, Hauffe & Pialek 1997). WARTs are considered rare in mammals. They are thought to result in complex meiotic pairing configurations (such as chromosomal rings or chains) when in the heterozygous condition, but detailed information on the expected underdominance associated with these types of rearrangements is scarce. When there is no additional information other than simply the presence of monobrachial homology (as it is the case in our study), it is impossible to distinguish between a WART on one hand, and a series of simple







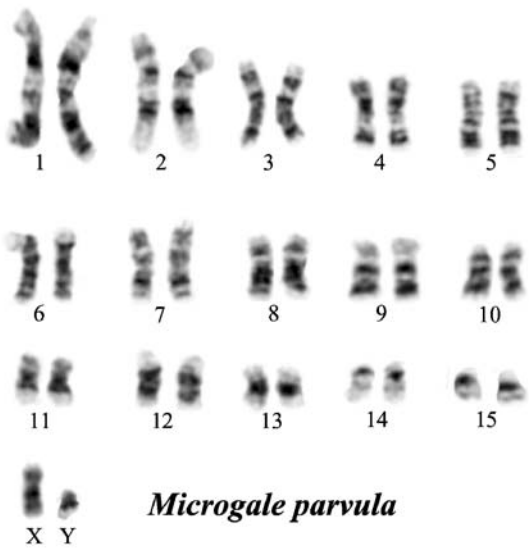


Figure 1. (continued)

Robertsonian (Rb) translocations on the other. The same pattern is expected after a WART between two metacentrics, and after two fissions of these metacentrics followed by two fusions of the resultant four acrocentrics. The fission/fusion hypothesis generally implies a greater number of steps and thus seems less parsimonious (Dobigny *et al*. 2004). However, since it has been shown that Rb translocations have a minimal impact on the fitness (for example in the house mouse; Nachman & Searle 1995), this class of rearrangement could, in spite of the greater number of steps, be considered more likely than WARTs. We have therefore erred on the side of caution and for this reason two character matrices were constructed.

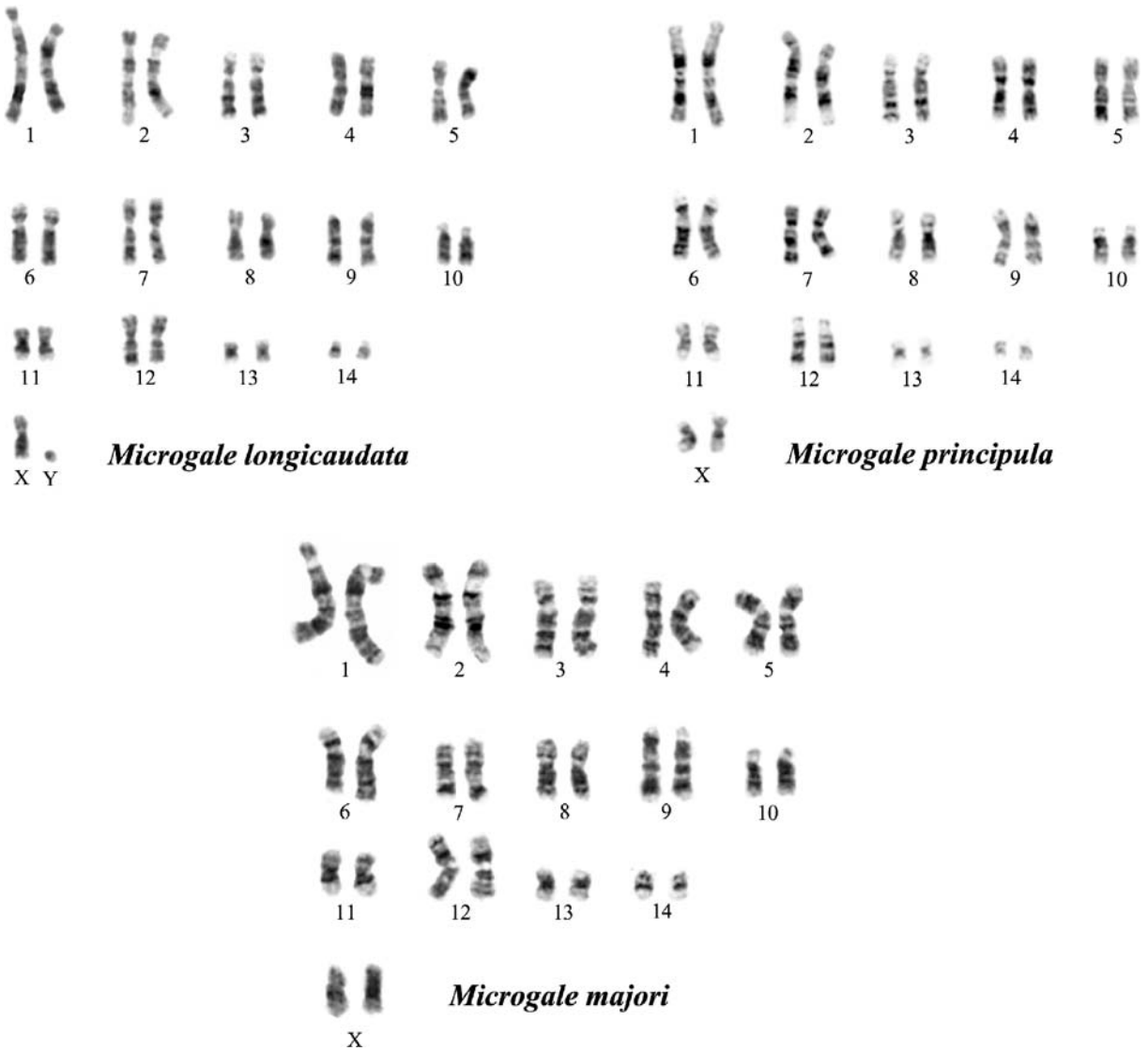


Figure 1. (continued)

In the first, all interchromosomal rearrangements were coded as fissions or fusions; in this case, WARTs, if present, were viewed to have resulted from two fissions followed by two fusions. In the second matrix, WARTs were coded as such (i.e., one step) wherever possible. The results obtained under these two hypotheses of chromosomal evolution are critically discussed and compared to other studies in order to determine whether one hypothesis received greater support than the other in our analyses. The two matrices are provided in Table 2. The most parsimonious tree inferred from each matrix was retrieved using an exhaustive search in PAUP 4.0b10 (Swofford 2002). Bootstrap analyses were performed using 1000 replicates of the original matrices.

Results and discussion

G-banded karyotypes of 11 species of the Oryzorictinae are presented in Figure 1. They represent the first banded karyotypes published for the Tenrecidae. Diploid numbers vary from 30 to 56 with five species characterized by 2n = 32 (*M. fotsifotsy*, *M. parvula*, *M.*

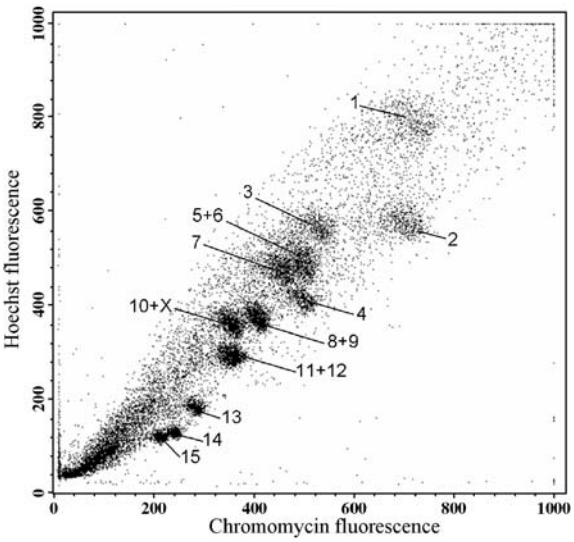


Figure 2. Flow-sorted karyotype of *Microgale taiva* FMNH 178756 (MTA, 2n = 32, XY) showing the correspondence between the peaks and MTA chromosomes (see text for details).

soricoides, *M. taiva* and *O. hova*), four with 2n = 30 (*M. dobsoni*, *M. longicaudata*, *M. majori* and *M. principula*), one with 2n = 38 (*M. cowani*), and one having 2n = 56 chromosomes (*M. thomasi*). Although an early report by Borgaonkar & Gould (1968) confirms the 2n = 30 recorded by us for *M. dobsoni*, in a subsequent paper these authors document a 2n = 54 for *M. cowani* (Borgaonkar & Gould 1969), a diploid number that differs markedly from the 2n = 38 observed in the present study. The ambiguity is compounded by the fact that no voucher specimens were collected or reported by these authors. In many ways, *M. cowani* exemplifies the complicated taxonomic history of shrew tenrecs; MacPhee (1987) synonymized five nominal species and one subspecies with *M. cowani*, two of which (*M. taiva* and *M. drouhardi*) have since been resurrected (see Jenkins 2003). Given the absence of a preserved voucher, the identification of the specimen karyotyped by Borgaonkar & Gould (1969) cannot be confirmed, and the notable difference in 2n between their specimen and FMNH 194138 (present study) will remain a mystery.

Although diploid numbers most commonly varied between 2n = 30 and 2n = 32 in the species examined, it was nonetheless often difficult to establish chromosomal homologies among them and the more rearranged (2n = 38 and 2n = 56) karyotypes using only the G-banding patterns. Consequently, chromosome painting using the flow-sorted chromosomes of *M. taiva* as painting probes was implemented to clarify homologies, and to identify complex rearrangements among species. Figure 2 shows the flow-karyotype of a male *M. taiva* specimen (MTA). The 30 chromosomes were resolved into 12 peaks. Eight peaks each contained a single chromosome pair (MTA 1–3, 4, 7, 13–15) and four peaks each contained two chromosome pairs (MTA 9 + 8, 5 + 6, 11 + 12, X + 10). The complete resolution of all 15 chromosome pairs was thus not possible, but this suite of painting probes in conjunction with the G-bands was sufficient to confidently resolve all chromosomal homologies among the tenrecids examined herein.

Ancestral karyotypes of Microgale, Oryzorictes *and the Oryzorictinae*

Chromosomal homologies between the 10 *Microgale* species and *O. hova*, a representative of the closely-related genus *Oryzorictes*, are illustrated in Figure 3. All the homologies are supported by chromosome painting data, several examples of which are presented in Figure 4. A striking result to emerge from these comparisons is that not a single interchromosomal rearrangement was detected between *M. taiva, M. parvula* and *O. hova* (see Figure 4a–e) underscoring their karyotypic conservatism since common ancestry. Moreover, the G-banding patterns are rather well conserved, suggesting little internal rearrangement within chromosomes. These data suggest, therefore, that the common ancestor of *Oryzorictes* and *Microgale* had a karyotype that was virtually identical to that observed in these three extant species, both with respect to diploid number (2n = 32) and G-banding pattern. Interestingly, the

Figure 3. G-banded half-karyotype comparison between 11 species of the Oryzorictinae showing the genome-wide chromosomal correspondence defined by painting and banding homologies. OHO = *Oryzorictes hova*; MTA = *Microgale taiva*; MPA = *M. parvula*; MDO = *M. dobsoni*; MMA = *M. majori*; MLO = *M. longicaudata*; MPR = *M. principula*; MSO = *M. soricoides*; MFO = *M. fotsifotsy*; MCO = *M. cowani*; MTH = *M. thomasi*. Closed circles indicate chromosomes that have undergone intrachromosomal rearrangements. Chromosome numbers are indicated for *M. taiva* and for the rearranged chromosomes of the other species in order to facilitate the correspondence with the diploid karyotypes (Figure 1).

Oryzorictes hova
Microgale talazaci
Microgale thomasi
Microgale cowani
Microgale fotsifotsy
Microgale soricoides
Microgale principula
Microgale longicaudata
Microgale major
Microgale dobsoni
Microgale parvula
Microgale taiva
OHO MTA MPA MDO MMA MLO MPR MSO MFO MCO MTH

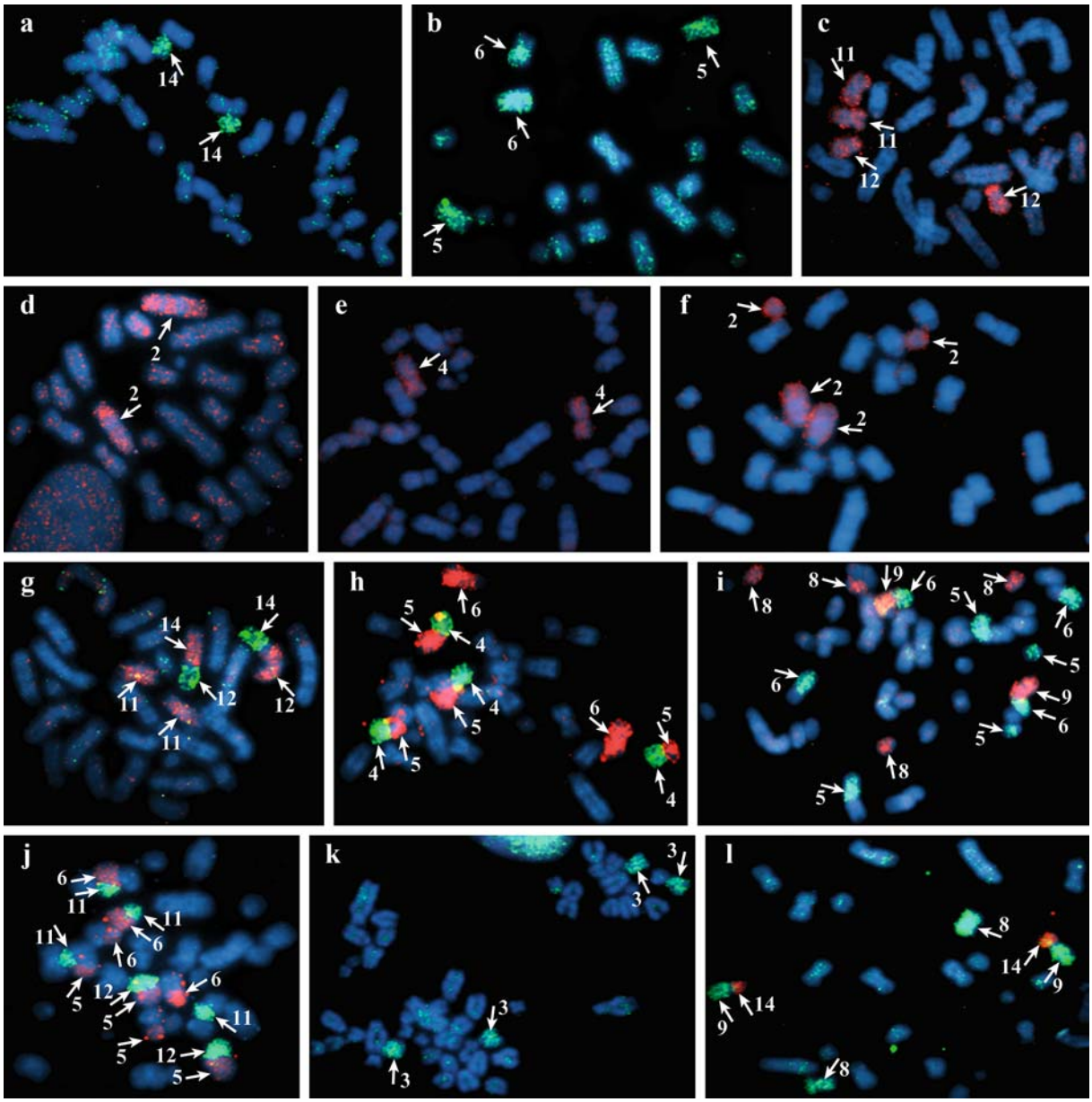


Figure 4. Examples of FISH using *Microgale taiva* (MTA) chromosome-specific painting probes. White arrows highlight the chromosomes of interest on all panels. Numbers refer to MTA chromosomes. Panels (a), (b), (c), (d), (e) present FISH of MTA 14, 5/6, 11/12, 2 and 4 respectively on metaphase of *Oryzorictes hova* showing that no interchromosomal break occurred in these chromosomes between *M. taiva* and *O. hova*. As illustrated by the following panels, these chromosomes are, however, all rearranged in other *Microgale* species. Panel (f) shows that MTA 2 has undergone a fission in *M. soricoides*. The same pattern was observed in *M. fotsifotsy*, *M. cowani* and *M. thomasi*. Panel (g) shows that MTA 14 (green) is fused with MTA 12 (red) in *M. longicaudata*. The same pattern was observed in *M. principula* and *M. majori*. Panel (h) illustrates the monobrachial homologies of MTA 4 (green) and MTA 5 (red) observed in *M. fotsifotsy*. MTA 6 (red) is not rearranged in this species. The same pattern was observed in *M. soricoides*. Panel (i) illustrates monobrachial homologies of MTA 9 (red) and 6 (green) observed in *M. cowani* and the fission of MTA 8 (red). The fission of MTA 8 was also observed in *M. thomasi*. Panel (j) illustrates monobrachial homologies of MTA 5 (red) and 12 (green) and of MTA 6 (red) and 11 (green) observed in *M. cowani*. Panel (k) shows that MTA 3 has undergone a fission in *M. thomasi* and panel (l) shows that MTA 9 has been fused to MTA 14 in *M. dobsoni*.

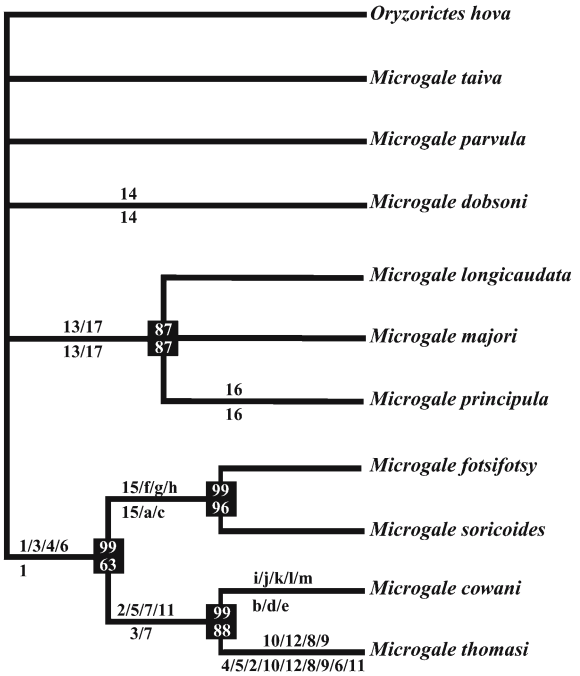


Figure 5. Single most parsimonious cladogram obtained after analysis of the two matrices presented in Table 2. Numbers and letters on branches refer to characters described in Table 2a (WART; bottom of the branches) and 2b (fusions/fissions; top of the branches). Bootstrap values based on the analysis of the two matrices are given at each node (Table 2a, bottom; Table 2b, top). Both matrices are homoplasy free (consistency indexes = 1).

ancestral karyotypes of the two genera *Oryzorictes* and *Microgale* are also likely to reflect the ancestral karyotype of the subfamily Oryzorictinae because the only other genus of this subfamily, the monotypic *Limnogale mergulus* is nested within the genus *Microgale* (Olson & Goodman 2003). It follows, therefore, that all rearrangements detected within *Microgale* can consequently be polarized using these inferred ancestral karyotypes as the outgroup. It is not possible to undertake a detailed comparison of the karyotypes described here with those of other tenrecids since only limited data are available for these taxa (i.e., unbanded karyotypes or only the diploid numbers). Interestingly, however, it is possible to infer that the oryzorictine ancestral karyotype is not found outside Oryzorictinae since all non-oryzorictine tenrecids have diploid numbers that differ from the 2n = 32 of the ancestral oryzorictine (Borganonkar 1967, Borganonkar & Gould 1965, Bernischke 1969).

WARTs vs. *fissions/fusions only*

Chromosomal changes detected in 12 of the 15 *M. taiva* (MTA, 2n = 30) chromosomes (Figure 3) were polarized as detailed above and coded as either present or absent in order to infer interspecific relationships within the genus *Microgale* (Table 2). Irrespective of whether WARTs were included to explain differences in chromosomal states between the species, or excluded in favour of the alternative hypothesis of Robertsonian (Rb) translocations, the same single most parsimonious tree was obtained in both instances (Figure 5). The length of the 'WART tree' was three steps shorter than that of the 'Rb tree' (22 vs 25 steps). Both matrices were homoplasy free as inferred by the high consistency indexes (CI = 1 for all characters and both matrices), but bootstrap values increased when only Rb translocations were considered. These findings are strikingly different from those obtained for the house mouse races from the Raethian Alps of northern Italy and southern Switzerland (Hauffe & Pialek 1997), as well as those from the Island of Madeira (Britton-Davidian *et al.* 2005). The inclusion of WARTs in these studies resulted in tree topologies that were not only different from those based on Rb translocations but were also much more parsimonious, being characterized by a maximum of nine (in Alpine mice) and five (in Madeiran mice) mutational steps fewer than were retrieved using the Rb translocations data. Additionally, the inclusion of WARTs reduced the level of homoplasy from a maximum of eight convergent events to only one in Madeiran mice, and increased support for all nodes (as measured by bootstrap and Bremer decay indexes) (Britton-Davidian *et al.* 2005) in this population, both trends that contrast strongly with our analysis of the tenrec data. Thus, while these observations strongly suggest that WARTs, in addition to Rb translocations, occurred in the house mouse (Hauffe & Pialek 1997, Britton-Davidian *et al.* 2005, Pialek *et al.* 2005), this clearly calls for a more detailed analysis in *Microgale*. The fact that the analysis of the Rb translocations data matrix resulted in an increase in the bootstrap values for the *Microgale* species' nodes does not appear sufficient in itself to favour this hypothesis. This increase is simply the result of a more homogeneous distribution of the rearrangements along the branches of the tree (Figure 4), and there is

no *a priori* justification for choosing this above the heterogeneous distribution evident when testing the WART hypothesis. Interestingly, however, in the case of the house mouse, the WART hypothesis is upheld by the fact that the fissions of Rb metacentrics are thought unlikely since telomeric and large amounts of centromeric satellite sequences are lost during Rb fusions in this species, and thus the subsequent fission of these Rb metacentrics would lead to acrocentrics deficient in these sequences (Garagna *et al.* 1995, Nanda *et al.* 1995). By contrast, fissions seem to be likely in *Microgale* as they are the most common rearrangements detected in our study. All chromosomes potentially involved in WARTs are also fissioned in at least one other species (Figure 3). This finding, together with the fact that WARTs (especially those of types b and c) are considered to be highly detrimental when in the heterozygous condition (Hauffe & Pialek 1997), would tend to support the observation that what holds true for the house mouse does not apparently hold for *Microgale*. Put succinctly, WARTs are much less likely to have occurred in *Microgale* than is the case with the house mouse.

Interspecific relationships within Microgale

Both matrices were homoplasy free (see above), resulting in four species clades with generally high bootstrap values ($BP_W = BP_{WARTs}$; $BP_{fi/fu} = BP_{fusion/fission}$) despite the inclusion of a relatively low number of characters (Figure 5). The first node groups *M. longicaudata*, *M. majori* and *M. principula* ($BP_W = 87$; $BP_{fi/fu} = 87$) on the basis that they share one fusion and one intrachromosomal rearrangement. The second node recovers *M. fotsifotsy* and *M. soricoides* as sister taxa ($BP_W = 96$; $BP_{fi/fu} = 99$), supported by either four fusions or one fusion and two WARTs (depending on which matrix is considered in the analysis), and the third groups *M. cowani* and *M. thomasi* ($BP_W = 88$; $BP_{fi/fu} = 99$) on the basis of either four or two shared fissions. Finally, the last node clusters the *fotsifotsy* + *soricoides* lineage as sister to *cowani* + *thomasi* ($BP_W = 63$; $BP_{fi/fu} = 99$), an association supported by either one or four fissions (depending on the matrix used).

These groupings are in perfect agreement with the topology obtained by Olson & Goodman (2003) derived from parsimony analysis of mitochondrial and nuclear gene sequences. In addition, the recognition of *M. longicaudata* and *M. principula* as sister species is supported by the parsimony analysis of morphological characters (Olson & Goodman 2003) and, interestingly, both (*M. soricoides* + *M. fotsifotsy*) and (*M. longicaudata* + *M. principula*) groupings correspond to distinct phenetic clusters based on overall similarities in their craniodental morphology, and the proportions thereof (MacPhee 1987, Jenkins 1993, Jenkins *et al.* 1997). It is important to note that *M. majori* was not considered in these studies given that it has only recently been resurrected from synonymy with *M. longicaudata* (based on molecular and morphometric analyses of a large number of specimens, see Olson *et al.* 2004). Unfortunately, the chromosomes are not informative in this respect since *M. majori* and *M. longicaudata* are karyotypically identical at the level of resolution permitted by their G-band patterns. However, the G-banded pattern of the chromosome resulting from the fusion of MTA 12 and 14 clearly differentiates *M. principula* from *M. majori* and *M. longicaudata* (Figure 3), representing as it does an autapomorphy for *M. principula*.

Finally, the last of the chromosomally distinct lineages, that of *M. dobsoni* (Figure 5), is characterized by a fusion between MTA 9 and 14 which represents an autapomorphy for this species. Although not informative in our tree, this character may prove to be phylogenetically important in future studies involving other *Microgale* species. In particular it will be interesting to see whether this rearrangement is present in *M. talazaci*, which is phenotypically (MacPhee 1987) and genetically (Olson & Goodman 2003) thought most closely associated to *M. dobsoni*.

Rates of chromosomal evolution within the Oryzorictinae

The subfamily Oryzorictinae includes the Malagasy tenrecs *Limnogale*, *Microgale* and *Oryzorictes*; *Limnogale* has, however, recently been considered to be nested within *Microgale* (see Olson & Goodman 2003). Poux *et al.* (2005) provide a molecular date for the *Oryzorictes* and *Limnogale* divergence (and thus an estimate for the Oryzorictinae). They calculate the divergence at 18.9 Myr (credibility interval = 14.1–24.7) using sequences from the nuclear exonic ADRA2B, AR and vWF gene fragments. Although *M. brevicaudata* was sequenced in the Poux *et al.*

study, the species was not included in their dating analysis because of missing vWF sequence. Since *Limnogale* is nested within *Microgale*, the analysis of sequences from *M. brevicaudata* as well as *L. mergulus* provides a means for dating the origin of *Microgale*, and thus a more refined timeframe for the discussion of the rates of chromosomal evolution within Oryzorictinae. We therefore repeated Poux *et al.*'s (2005) analysis using their sequence matrix (available in Treebase; accession number M2279) and the same criterion for discarding ambiguous regions in the alignment, the same calibration points and identical Bayesian methods (which can handle missing data, see Thorne & Kishino 2002), but including *M. brevicaudata* (i.e., the ADRA2B and AR sequences that were not analysed in the original study). We estimate the *L. mergulus*/*M. brevicaudata* split at ~9.9 Myr (Cred. Int. = 6.3–14.8), which can be interpreted as a minimum age for *Microgale*. To place our discussion in context, it is important to emphasize (i) that no interchromosomal change occurred during the ~18.9 Myr that separates *O. hova* from the oryzorictine ancestor (Poux *et al.* 2005), and (ii) that chromosomal stasis similarly characterizes both the 9 Myr (i.e., 18.9–9.9 Myr) separating the oryzorictine ancestor from the *Microgale* ancestor, and the ~9.9 Myr distinguishing the *Microgale* ancestor from *M. taiva* and *M. parvula*. Moreover, during the same period (i.e., ~9.9 Myr), only one interchromosomal change was detected on the lineage leading to *M. dobsoni* and two were detected on the lineage leading to *M. longicaudata*, *M. majori* and *M. principula* (Figure 5). These observations are in keeping with the low rates that have been reported in the Chrysochloridae (Gilbert et al. 2006), sister family to the Tenrecidae, and are consistent with a more generalized slow rate for the Afroinsectiphillia (Afrosoricida + Macroscelidae + Tubulidentata). In contrast, 12 chromosomal changes detected in *M. thomasi* and between 6 and 13 (depending on which matrix is considered) in *M. cowani* punctuate the ~9.9 Myr that separate these two species from the *Microgale* ancestor (Figure 5), mimicking the karyotypic megaevolution of certain bat species (Baker & Bickham 1980). Although these rates are lower than those observed in several mammals (e.g., Britton-Davidian *et al.* 2000, Wang & Lan 2000, Dobigny *et al.* 2005), they are clearly accelerated with respect to most Afrotheria, the only exception being within the Sirenia where at least four chromosomal changes separate *Trichechus inunguis* and *T. manatus*, taxa that are thought to have diverged 1–4 Myr ago (Pardini *et al.* 2007). Chromosomal mutation rates depend on many factors including population dynamics, life history and genomic traits of the species concerned. It will be interesting to see which of these is more likely to account for the large rate differences observed between species such as *M. thomasi* (12 changes in 9.9 Myr) *cf. M. taiva* (no change in 9.9 Myr) for example. Unfortunately, other than suggesting their usefulness as models for testing the effects of differing life histories and other biological traits on rates of chromosomal change, the paucity of data on these species precludes any meaningful comparisons.

Chromosomal speciation in Microgale

The tenrec species included in our study all occur in sympatry in the humid forests of the Central Highlands of Madagascar (Goodman & Rakotondravony 2000, Jenkins 2003 and references therein). Contemporary distributions do not, however, necessarily reflect the ancestral condition, requiring that temporal, as well as climatic aspects must be considered in any discussion of the potential causes of speciation in a specific group of taxa. Wilmé *et al.* (2006) have recently provided a compelling biogeographic model to explain the high number of speciation events that the extant vertebrate fauna of Madagascar has undergone. These authors suggested that during the Quaternary glacial maxima, when climatic conditions were cooler and drier and animals sought refuge in more mesic riverine forest, watersheds with their sources at lower elevations would have been dispersal dead ends and areas in which extensive allopatric speciation could have occurred. In addition, a recent study by Olivieri *et al.* (2007) involving a comprehensive taxonomic sample of mouse lemur species (*Microcebus*) argued that factors such as ancestral distribution, species-specific habitat preference, as well as the role of rivers and mountains as barriers to gene flow (initially proposed by Martin 1972, 1995) are fundamental to understanding the diversification and present distribution of mouse lemurs.

Interestingly, our estimated minimum age for the origin of *Microgale* (9.9 Myr [Cred. Int. = 6.3–14.8]) is close to that calculated for the lemur genera *Microcebus* (8.9 Myr [Cred. Int. = 5.5–13.2]) and

Eulemur (9.7 Myr [Cred. Int. = 6.5–13.7]) (Yoder & Yang 2004). Although the age of *Microgale* might here be underestimated, and may be refined through greater taxonomic representation, it nonetheless shows that the evolutionary histories of *Microcebus*, *Eulemur* and *Microgale* are largely concordant, and thus that the mechanisms that have shaped the diversification of lemurs might have also influenced the evolutionary history of shrew tenrecs. Should this hold, the observed sympatric patterns exhibited by most species of shrew tenrecs are the result of secondary contact that occurred subsequently to allopatric speciation.

Although chromosomal speciation is not ubiquitous in *Microgale* (two sets of well-defined species, *M. taiva*/*M. parvula*, and *M. fotsifotsy*/*M. soricoides*, have an identical karyotype), a causal role for chromosomal rearrangements in speciation is plausible where marked differences in karyotypes are found. When inferring a causal mechanisms of speciation it is clearly necessary to ascertain that it is really pairs of sister species where we find such distinct chromosomal differences and, at this point, there are no data on geographic karyotypic variation and no independent, reliable information on the identity of definitive sister species in this genus. In spite of these limitations, however, it is not unreasonable to suggest that the extensive chromosomal rearrangements detected in our study may have driven speciation in *Microgale* through the negative effects of underdominance (heterozygote meiotic breakdown), (White 1968, Baker & Bickham 1986, King 1993; see Rieseberg 2001 for critical discussion), forming as it does, an hypothesis that can be tested empirically in subsequent studies.

In most models, it is generally assumed that chromosomal rearrangements must be somewhat deleterious in the heterozygous condition, requiring extreme conditions for their fixation including small population size and inbreeding among others (the monobrachial homology model of Baker & Bickham 1986 being an exception). These are conditions that could quite plausibly have existed under the Wilmé *et al*. model, at least during the climatic shifts of the Pliocene/Pleistocene, during which populations of a previously widespread species of *Microgale* may have been isolated in several low-elevation watersheds in a glacial maximum. During the subsequent glacial minimum, heterozygote hybrids resulting from crosses between specimens from previously isolated populations could have exhibited reduced fertility or, in extreme instances, complete reproductive breakdown. The complex meiotic configurations anticipated to result from the multiple rearrangements that define many of the species examined herein (chains and/or rings of chromosomes) could reasonably be expected to result in malsegregation and/or germ cell death.

In the case of the house mouse races, hybrid fertility varies considerably with the number of heterozygous rearrangements present in carriers (Nachman & Searle 1995). In some instances a single heterozygous rearrangement may be sufficient

Table 3. Number and type of abnormal meiotic configurations expected in all possible hybrids resulting from theoretical crossings of any pair of chromosomally different *Microgale* species included herein (based on Figure 3). Only interchromosomal rearrangements are considered

	MTA,MPA	MSO,MFO	MPR,MMA,MLO	MTH	MCO
MSO,MFO	1 chain of 3 1 chain of 5 1 ring of 4				
MPR,MMA,MLO	1 chain of 3	1 chain of 3 2 rings of 4			
MTH	10 chains of 3 1 chain of 4	11 chains of 3 1 chain of 4	11 chains of 3 1 chain of 4		
MCO	3 chains of 3 1 ring of 4 1 chain of 4 1 chain of 6	1 chain of 9 3 chains of 3 1 chain of 6 1 chain of 4	3 chains of 3 1 chain of 5 1 chain of 6 1 ring of 4	5 chains of 3 2 chains of 4	
MDO	1 chain of 3	1 chain of 3 1 chain of 6 1 ring of 4	1 chain of 4	9 chains of 3 2 chains of 4	3 chains of 3 1 chain of 4 1 chain of 7 1 ring of 4

to adversely affect fertility (Hauffe & Searle 1998), while in others those carrying few heterozygous rearrangements do not generally show a decrease in fertility (Winking *et al*. 1988, Viroux & Bauchau 1992, Wallace *et al*. 1992). On the contrary, however, hybrids heterozygous for more than three rearrangements generally show elevated levels of aneuploidy and/or germ cell death (Redi & Capanna 1978, Garagna *et al*. 1990, Saïd *et al*. 1993). It is also anticipated that meiotic chains are more detrimental than rings of chromosomes since they present unpaired axes and the more meiotic abnormal configurations present, the less fertile the hybrid (Hauffe & Pialek 1997). We determined the number of complex meiotic configurations theoretically expected in hybrids that would result from crosses among the chromosomally distinct shrew tenrec species identified by our investigation (Table 3). Nine of the possible 15 interspecific crosses would result in hybrid meiosis characterized by a high number of chains and/or rings (between 6 and 11 abnormal pairing configurations per specimen), an observation that warrants further detailed empirical analysis, among others, through captive breeding experiments. We recognize that it could be argued that the final arbiter of correct segregation is the meiotic spindle (Eichenlaub-Ritter & Winking 1990, King 1993) and that not all instances of shrew tenrec hybridization depicted in Table 3 may have been possible (due to geographic or the development of other premating barriers before the possibility of secondary contact). Nonetheless, given Madagascar's paleoclimatic oscillations and the spectacular shrew tenrec species diversity which is often underpinned by marked differences among karyotypes, a case can be made for including *Microgale* in the suite of taxa (*Spalax* [Nevo *et al*. 1994], *Muntiacus* [Wang & Lan 2000], *Mus musculus domesticus* [reviewed in Capanna & Castiglia 2004], *Taterillus* [Dobigny *et al*. 2005] among others) for which the fixation of underdominant chromosomal rearrangements may have played a role in cladogenesis.

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