Comparing chromosomal and mitochondrial phylogenies of the Indriidae (Primates, Lemuriformes)

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Abstract The Malagasy primate family Indriidae comprises three genera with up to 19 species. Cytogenetic and molecular phylogenies of the Indriidae have been performed with special attention to the genus *Propithecus*. Comparative R-banding and FISH with human paints were applied to karyotypes of representatives of all three genera and confirmed most of the earlier R-banding results. However, additional chromosomal rearrangements were detected. A retic-ulated and a cladistic phylogeny, the latter including hemiplasies, have been performed. Cladistic analysis of cytogenetic data resulted in a phylogenetic tree revealing (1) monophyly of the family Indriidae, (2) monophyly of the genus *Avahi*, (3) sister–group relationships between *Propithecus diadema* and *Propithecus edwardsi*, and (4) the grouping of the latter with *Indri indri*, *Propithecus verreauxi*, and *Propithecus tattersalli*, and thus suggesting paraphyly of the genus *Propithecus*. A molecular phylogeny based on complete mitochondrial cytochrome *b* sequences of 16 species indicated some identical relationships, such as the monophyly of *Avahi* and the sister–group relationships of the eastern (*P. diadema* and *P. edwardsi*) to the western *Propithecus* species (*P. verreauxi*, *Propithecus coquereli*, and *P. tattersalli*). However, the main difference between the molecular and cytogenetic phylogenies consists in an early divergence of *Indri* in the molecular phylogeny while in the chromosomal phylogeny it is nested within *Propithecus*. The similarities and differences between molecular and cytogenetic phylogenies in relation to data on the species' geographic distributions and mating systems allow us to propose a scenario of the evolution of Indriidae. Chromosomal and molecular processes alone or in combination created a reproductive barrier that was then followed by further speciation processes.

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Abbreviations

ALA	*Avahi laniger*
AOC	*Avahi occidentalis*
CCD	Charge-coupled device
DAPI	4′,6-diamidino-2-phenylindole
DMSO	Dimethylsulfoxide
EFU	*Eulemur fulvus*
FISH	Fluorescence *in situ* hybridization
FITC	Fluorescein isothiocyanate
h	Heterochromatin
HSA	*Homo sapiens*
IIN	*Indri indri*
ML	Maximum likelihood
MMR	*Microcebus murinus*
MP	Maximum parsimony
mtDNA	Mitochondrial DNA
NJ	Neighbor joining
PAUP	Phylogenetic analysis using parsimony
PCO	*Propithecus coronatus*
PCQ	*Propithecus coquereli*
PDI	*Propithecus diadema*
PED	*Propithecus edwardsi*
PTA	*Propithecus tattersalli*
PVE	*Propithecus verreauxi*

Introduction

The island of Madagascar is famous for its high rate of endemic plant and animal species and for its species richness. Around 100 endemic primate species are described (Mittermeier et al. 2010), all of which are descendants from a single ancestral population that colonized the island in the early Eocene (Horvath et al. 2008; Roos et al. 2004). Although knowledge about the diversity and evolution of Madagascar's primate species has increased in the last decades, detailed information for most taxa is still missing. This is especially true for members of the family Indriidae. While there is consensus that the family contains the three genera, *Indri*, *Propithecus*, and *Avahi*, the number of species to be recognized and phylogenetic relationships among them remains disputed.

Among the three genera, the nocturnal woolly lemurs of the genus *Avahi* are the smallest taxa (~1 kg). Originally only one species, *Avahi laniger* ($2n=70$), with an eastern and western subspecies was recognized (Schwarz 1931; Petter et al. 1977; Tattersall 1982).

Subsequently, both subspecies were elevated to full species, *A. laniger* and *Avahi occidentalis* by Rumpler et al. (1990) based on cytogenetic characters. Later, two additional species, *Avahi unicolor* and *Avahi cleesei*, were described based on morphological characters by Thalmann and Geissmann (2000, 2005). Recent molecular investigations further increased the number of species to seven (Zaramody et al. 2006) and even to nine (Andriantompohavana et al. 2007; Lei et al. 2008). Although the taxonomic status of several of these forms remains disputed (Zaramody et al. 2006), we follow here the current classification with nine *Avahi* species (Mittermeier et al. 2010) (for species ranges, see Fig. 1).

Sifakas, genus *Propithecus*, are diurnal and larger than woolly lemurs. The genus originally contained two species, the smaller *Propithecus verreauxi* (3–4.5 kg) with four subspecies (*P. v. coquereli*, *P. v. coronatus*, *P. v. deckeni*, *P. v. verreauxi*) from the western and southern dry forests, and the larger *P. diadema* (5–8.5 kg) with five subspecies (*P. d. candidus*, *P. d. diadema*, *P. d. edwardsi*, *P. d. holomelas*, *P. d. perrieri*) from eastern Madagascar (Hill 1953; Petter et al. 1977). Tattersall (1986), however, regarded *P. d. holomelas* as synonym of *P. d. edwardsi*, while Simons (1988) added *P. tattersalli* as an additional species. In recent classifications, all previous subspecies of *P. diadema* (Mayor et al. 2004) and *P. verreauxi* were elevated to distinct species, resulting in a total of nine species (Mittermeier et al. 2010).

For the *P. diadema* group, cytogenetic investigations distinguished between two karyotypes ($2n=42$ for *P. diadema*, *P. candidus*, and *P. perrieri*, and $2n=44$ for *P. edwardsi*), while all members of the *P. verreauxi* group carry the same diploid chromosome number of $2n=48$ (Rumpler and Albignac 1979; Rumpler et al. 2004). *P. tattersalli* shows a diploid chromosome number of $2n=42$ (Rumpler et al. 2004). Based on mitochondrial sequence data, three major clades among sifakas were detected (Razafindraibe et al. 2000; Pastorini et al. 2001; Mayor et al. 2004). The first comprises all members of the *P. diadema* group, in the second *P. tattersalli* and *P. coquereli* cluster together, and the third contains the remaining members of the *P. verreauxi* group.

In contrast to the former two genera, the genus *Indri* is monotypic comprising the single species *Indri indri* ($2n=40$). Indris are the largest extant lemurs (6–9.5 kg) and occur only in the central to northern parts of the eastern Malagasy forest with a range extending
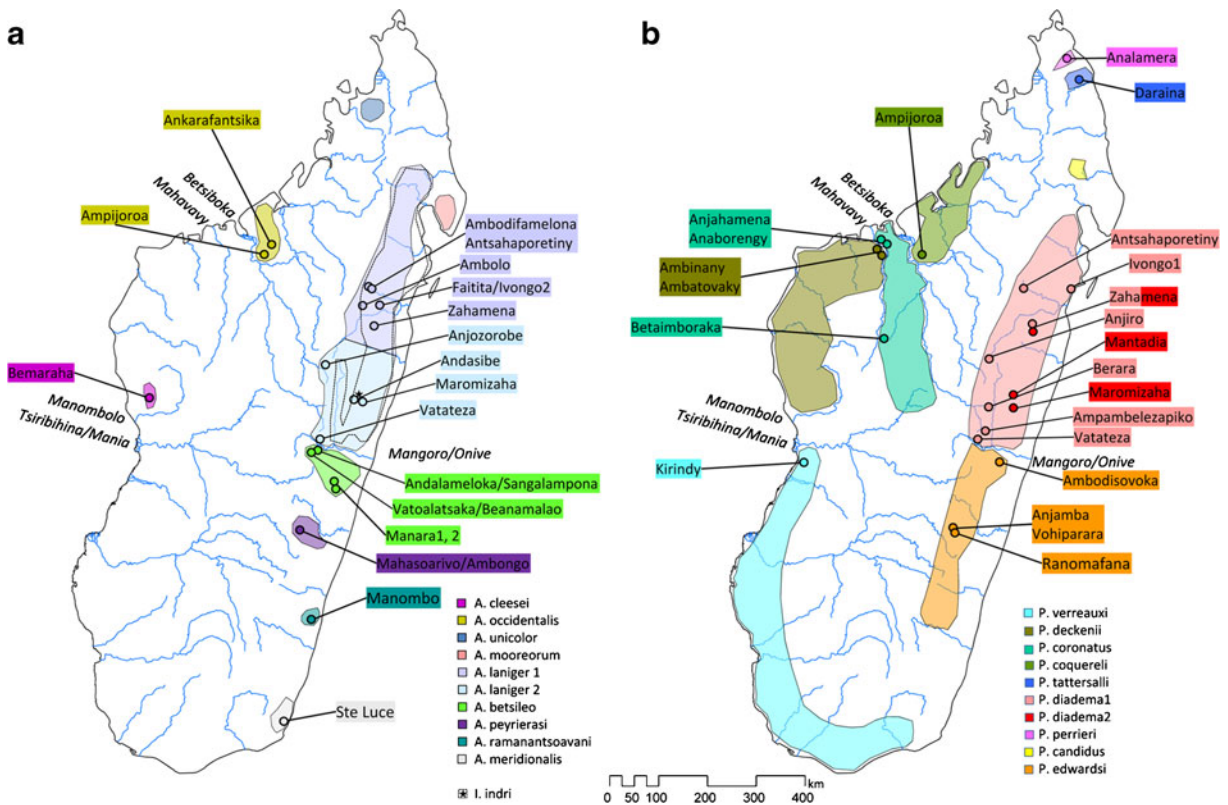
Fig. 1 Simplified distribution maps of **a** *Avahi/Indri* and **b** *Propithecus*. Also indicated are sampling locations, as mentioned in Supplementary Table S1. Distribution maps modified from Lei et al. (2008) and Mittermeier et al. (2010)

from the Anosibe an'ala Classified Forest in the south to the Anjanaharibé-Sud Special Reserve in the north (Petter et al. 1977; Tattersall 1982; Powzyk and Thalmann 2003). This species is well characterized by both karyotypic and molecular data (Rumpler et al. 1988; Delpero et al. 2001; Roos et al. 2004).

Although chromosomal (Rumpler et al. 2004) and mitochondrial sequence data (Pastorini et al. 2001; Mayor et al. 2004; Roos et al. 2004; Zaramody et al. 2006) are available for several Indriidae species, all these studies are characterized by an incomplete taxonomic and geographic sampling, especially for *Propithecus*. Moreover, some taxa have been split into several species within the last years and therefore might have been misidentified in earlier studies. To obtain a more complete picture of the evolution of this enigmatic primate family, information of recently discovered species and samples with known geographic provenance are necessary. Furthermore, Rumpler et al. (2004) proposed that the most parsimonious model for chromosomal evolution in Indriidae would include a

phase of reticulation. In light of possible hemiplasic relationships in chromosomal phylogenies, as suggested by Robinson et al. (2008) and Avise and Robinson (2008), it is necessary to explore whether reticulation is still the most parsimonious model.

In our study, we analyze the phylogenetic relationships between the species of the three indriid genera by cladistic methods using cytogenetic data derived from comparative R-banding and fluorescence *in situ* hybridization (FISH) with human paints. We establish a cladistic phylogeny and compare it with the model of reticulated chromosomal evolution proposed in an earlier study (Rumpler et al. 2004). Furthermore, we establish a molecular phylogeny based on mitochondrial cytochrome *b* (cyt *b*) sequence data from all three indriid genera and most of their species, and compare it with the chromosomal phylogenetic patterns. This enables us to propose an evolutionary scenario based on phylogenetic analyses of molecular and cytogenetic data in combination with data on the species' geographic distributions. This allows us also

to refine the distributions of parapatric *Propithecus* species pairs (*P. diadema*/*P. edwardsi* and *P. coronatus*/ *P. deckeni*) (Curtis et al. 1998; P. Moisson, personal communication).

Methods

Samples

For *Propithecus*, 55 tissue samples were collected during several field surveys (2002–2008) in different parts of the eastern forests of Madagascar. Samples were collected from Analamera in the north to Ranomafana in the south, and in the western forests from Ampijoroa in the north to Kirindy in the south (Fig. 1, Supplementary Table S1). Animals were caught and anesthetized with a 2 mg/kg injection of ketamine solution (Ketalar® Parke-Davis). Skin biopsies were taken under general anesthesia and frozen in liquid nitrogen after adding DMSO (10%) as cryoprotector. The samples were first kept at the Institute Pasteur of Antananarivo and then sent to the Institute of Embryology of the University of Strasbourg, France. The collection of samples was approved by the respective Malagasy authorities.

For the cytogenetic study, we used, in addition to the *Propithecus* samples, fibroblast cultures from *Avahi* species, *I. indri*, and the two outgroup species *Microcebus murinus* and *Eulemur fulvus* which were already present at the cell bank of the Institute of Embryology (Table 1). For the molecular analysis, we used all 55 *Propithecus* samples, one sample of *Indri* from the cell bank of the Institute of Embryology, and the respective data previously published by Zaramody et al. (2006) from 49 samples of *Avahi*. Latter samples were collected in different parts of the eastern Malagasy forests from Antsahaporetiny in the north to Fort Dauphin in the south and in the western forests from Ampijoroa and Bemahara (Fig. 1). No samples were available for *A. mooreorum*, *A. unicolor*, and *P. candidus*.

Cytogenetics

R-banding was performed on three samples of *P. diadema* from Ampambelezapiko, Ivongo, and Mantadia; four *P. edwardsi* from Ambodisovoka, Anjamba, and Vohipara; three *P. tattersalli* from

Table 1 Number of samples per taxon included in karyotyping and molecular analysis

Taxon	R-banding	Giemsa	FISH	cyt *b*
A. occidentalis	1	0	1	3
A. cleesei	1	0	0	1
A. laniger	4	4	1	18
A. peyrierasi	1	0	0	4
A. betsileo	0	0	0	6
A. ramanantsoavani	0	2	0	1
A. meridionalis	0	0	0	4
A. unicolor	0	0	0	0
A. mooreorum	0	0	0	0
P. diadema	3	2	1	14
P. perrieri	1	0	0	1
P. edwardsi	4	3	1	7
P. tattersalli	3	0	1	4
P. coquereli	1	0	1	4
P. verreauxi	1	0	1	3
P. coronatus	2	0	1	5
P. deckeni	0	1	0	6
P. spp.[a]	0	0	0	11
P. candidus	0	0	0	0
I. indri	1	0	1	1
Total	23	12	9	93

[a] Phenotypically *P. coronatus* × *P. deckeni* hybrids

Daraina; one *P. verreauxi* from Kirindy; one *P. coquereli* from Ampijoroa; and two *P. coronatus* from the Zoo of Mulhouse, France (Table 1). From all other Indriidae species, R-banded karyotypes were taken from Rumpler et al. (2004). For 12 samples which did not give enough mitosis to allow R-banding, only Giemsa staining was performed (Table 1).

FISH techniques were applied to the chromosomes of one sample of each of the following nine species: *P. verreauxi*, *P. coronatus*, *P. coquereli*, *P. diadema*, *P. edwardsi*, *P. tattersalli*, *I. indri*, *A. laniger*, and *A. occidentalis*. We tried to use a complete set of human chromosome paints for the different species, but when the repetitive fibroblast cultures of a species gave rise only to a reduced number of slides showing numerous metaphases with well-dispersed chromosomes, we chose not to paint chromosomes with similar R-banding pattern in closely related species. In detail, we used the following paints in the different species: HSA1, HSA12, HSA16, HSA17, and HSA19 for all species; HSA2, HSA3, HSA4, HSA6, HSA11,

HSA14, HSA15, and HSA21 for all species except *P. edwardsi*; HSA5 for all except *P. edwardsi* and *P. diadema*; HSA7 and HSA8 for all except *P. edwardsi* and *P. tattersalli*; HSA10 for all species except *A. occidentalis*; HSA13 for all except *A. occidentalis* and *P. diadema*; and HSA18, HSA20, and HSA22 for all except *P. edwardsi*, *P. tattersalli*, and *A. occidentalis*. FISH experiments were conducted using commercially available whole human (HSA) chromosome paints (Biosys SA—Cambio, Cambridge, UK) for all autosomes. *In situ* hybridization was performed as described in the manufacturer's instructions with some modifications (Vezuli and Rumpler 2000). After incubation for 72 h, slides were washed at 40°C. Biotinylated DNA probes were detected via avidin coupled with fluorescein isothiocyanate (FITC) and visualized without further amplification of the signal. Metaphases were counterstained with propidium iodide or DAPI. The hybridization signals were observed via an epifluorescence Zeiss Axioplan microscope and digital images were taken with a cooled CCD camera (JAI M300) linked to the microscope. As imaging software, Isis3 (MetaSystem GmbH, Germany) was used.

We reconstructed and compared a cladistic phylogeny and a phylogeny containing a reticulate phase. In the cladistic model, changes of the karyotypic structure between the different Indriidae and outgroup species were identified and then coded as binary (presence=1, absence=0) characters following the principles described in Dobigny et al. (2004). Only informative, i.e., different character states between outgroup taxa and at least one of the ingroup taxa, and autapomorphic characters were included in the matrix (Supplementary Table S2). As outgroup taxa, we used *M. murinus* (MMR, $2n=66$) and *E. fulvus* (EFU, $2n=60$) since both are representatives of two other Malagasy lemur families (Cheirogaleidae and Lemuridae, respectively), and since for both species detailed R- and C-banding (Rumpler and Dutrillaux 1990) as well as painted data are available. The same outgroups were chosen in an earlier study on sportive lemurs (Rumpler et al. 2008). Primary homologies were identified by comparing R-banding patterns and FISH data from the seven cytogenetically different Indriidae species (*A. laniger* $2n=70$, *A. occidentalis* $2n=70$, *P. tattersalli* $2n=42$, *P. diadema* $2n=42$, *P. edwardsi* $2n=44$, *P. verreauxi* $2n=48$, and *I. indri* $2n=40$) and the two outgroup taxa. Importantly, no *a priori* polarization (e.g.,

translocation vs. fission) was assumed and changes were all strictly inferred by outgroup comparison criteria, thus allowing an *a posteriori* reconstruction of chromosome evolution. We constructed a phylogenetic tree based on the binary coded chromosomal rearrangements with the maximum-parsimony (MP) algorithm where some reversions and/or convergence rearrangements are allowed. The MP tree was calculated via an exhaustive search in PAUP 4.0b10 (Swofford 2000) and robustness of internal branches was verified with 10,000 bootstrap replications.

In a second approach, we considered possible reversions or convergences as rearrangements that existed at an ancestral polymorphic stage which were only retained in some lineages while they were lost in others, thus leading to hemiplasic relationships (Avise and Robinson 2008; Robinson et al. 2008). The absence of such rearrangements, however, does not correspond to the reversion of a fixed rearrangement.

For the reticulate model, we manually reconstructed a hypothetical ancestral karyotype by comparing the karyotypes of the different species according to the method previously used by Dutrillaux (1986) and Rumpler et al. (2004). The respective phylogenetic tree, starting from this ancestral karyotype and leading to the different species, was constructed in the most parsimonious way without convergence or reversion. Consequently, rearrangements which appeared to be reversions or convergences in the cladistic reconstruction have to be exchanged by hybridization between diverging lineages. The question whether a reticulated evolution is *a priori* more likely to occur than reversions or convergences remains debatable. Indeed, there is clear evidence that convergences and reversions are common in chromosomal evolution. Such cases have been reported for several rodent species (Qumsiyeh et al. 1987; Volobouev et al. 2002; Britton-Davidian et al. 2005) and lemurs (Warter et al. 2005).

Molecular genetics

A molecular phylogeny was reconstructed with sequences of the complete mitochondrial cyt *b* gene (1,140 bp). Therefore, 38 unique sequences (37 for *Avahi*, one for *Indri*) taken from GenBank were combined with 55 newly generated *Propithecus* sequences (Supplementary Table S1). DNA extraction, PCR amplification, and sequencing followed methods as described in Roos et al. (2004) and

Andriaholinirina et al. (2006). Due to the absence of indels, the alignment was easily generated by eye. To exclude the possibility that nuclear pseudogenes (numts) were amplified, all sequences were checked for premature stop codons. The final Indriidae data set included 71 unique haplotypes representing 16 taxa.

Phylogenetic trees were constructed with maximum-likelihood (ML) and Bayesian algorithms using GARLI 0.951 (Zwickl 2006) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). As outgroup taxa, we used *Lepilemur ruficaudatus*, *Phaner furcifer*, *Lemur catta*, and *Daubentonia madagascariensis*, which were all available in GenBank. For both reconstructions, the HKY+I+Γ model was applied as it was chosen as best-fit model under the Akaike Information Criterion by MODELTEST 3.7 (Posada and Crandall 1998). In GARLI, only the model specification settings were adjusted according to the dataset, while all other settings were left at their default value. Support of internal branches was calculated with 500 bootstrap replications. ML majority-rule consensus trees were calculated in PAUP. For Bayesian reconstructions, we used four Markov Chain Monte Carlo chains with the default temperature of 0.1. Four repetitions were run for 10,000,000 generations with tree and parameter sampling occurring every 100 generations. The first 25% of samples were discarded as burnin, leaving 75,001 trees per run. Posterior probabilities for each split and a phylogram with mean branch lengths were calculated from the posterior density of trees.

Results

Cytogenetics

Recurrent signals were obtained for all hybridized chromosome painting probes (Fig. 2). The hybridization quality, however, was much lower than that seen for human chromosomes on the same slide. Among the 16 karyotyped taxa, only seven unique karyotypes were detected (Table 2): *A. occidentalis* (=*A. cleesei*), *A. laniger* (=*A. peyrierasi*=*A. ramanant-soavani*=*A. meridionalis*=*A. betsileo*), *P. diadema* (=*P. perrieri*), *P. edwardsi*, *P. tattersalli*, *P. verreauxi* (=*P. coronatus*=*P. deckeni*=*P. coquereli*), and *I. indri*. This result confirms and complements earlier findings from Rumpler et al. (1988), Mayor et al. (2004), and Zaramody et al. (2006).

In Table 3, homologous segments between the chromosomes of these karyotypes and the two outgroup taxa as obtained from R-banding techniques are listed. The FISH techniques mostly confirmed earlier results solely based on R-banding techniques (Rumpler et al. 2004), but allowed us to specify more precisely some other rearrangements and expanded results of earlier studies (Table 3).

Fig. 2 Example of homologous regions of human chromosomes HSA1 and HSA6 established by FISH. *Orange arrows* indicate HSA6 paints of PVE 1p and 11p; *white arrows* indicate HSA1 paints of PVE 19, 5q, 7p prox, and 21. *Scale bar* represents 10 μm
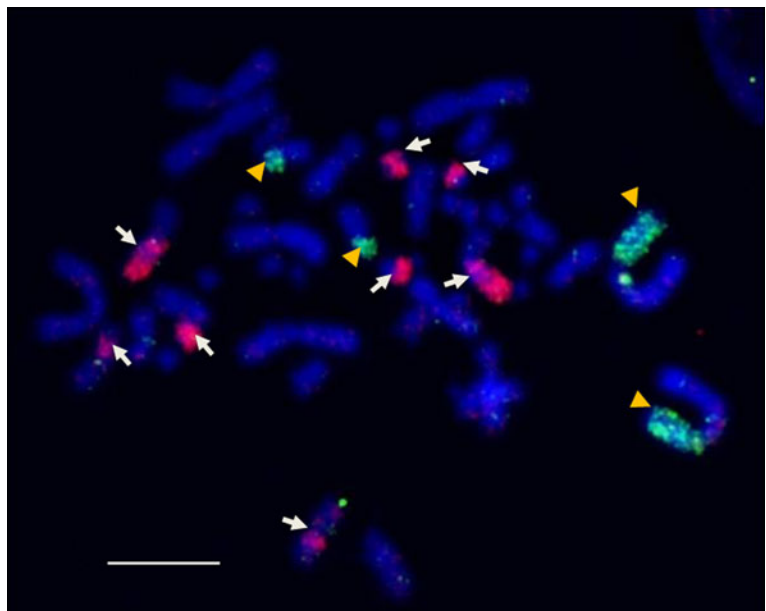
Table 2 Distribution of karyotypes in Indriidae species

Taxon	2N	Karyotype	Reference
A. occidentalis	70	1 AOC	1, 2 this study
A. cleesei	70	1 AOC	2, this study
A. unicolor	70	?	
A. laniger	70	2 ALA	2, 3, this study
A. peyrierasi	70	2 ALA	2, this study
A. ramanantsoavani	70	2 ALA	2, this study
A. meridionalis	70	2 ALA	2, this study
A. betsileo	70	2 ALA	2, this study
A. mooreorum		?	
P. diadema	42	3 PDI	1, 4, this study
P. perrieri	42	3 PDI	4, 5, this study
P. candidus	42	3 PDI	4
P. edwardsi	44	4 PED	1, 4, this study
P. tattersalli	42	5 PTA	1, 4, this study
P. verreauxi	48	6 PVE	1, 4, this study
P. coquereli	48	6 PVE	1, 4, this study
P. coronatus	48	6 PVE	1, this study
P. deckeni	48	6 PVE	5
I. indri	40	7 IIN	1, this study

Among the 16 analyzed species, only seven karyotypes were found. *P. candidus* was not karyotyped in our study, but Mayor et al. (2004) found it to be identical to *P. diadema*. References: *1* Rumpler et al. (2004), *2* Zaramody et al. (2006), *3* Rumpler et al. (1990), *4* Mayor et al. (2004), *5* Rumpler and Albignac (1979)

As a result, we present the following new cytogenetic data: one previously undetermined rearrangement involving three minute chromosomes to give rise to IIN10 has been replaced by one Robertsonian translocation followed by a centromere–telomere one [50] (numbers in squared parentheses refer to rearrangements as listed in Table 4 and are also identical to rearrangements in Figs. 3 and 4). In addition, one undetermined rearrangement giving PTA14 has been replaced by a Robertsonian translocation [49]. ALA3 which was the result of a complex rearrangement including two chromosomes finally appears as a result of three rearrangements [15,16,17]. Two minute chromosomes of *Avahi* which could not be identified in earlier studies are the result of a centromere–telomere fission [34]. On the other hand, two rearrangements [32] and [33] which were considered as common for *P. verreauxi*, *P. diadema*, and *Indri* and specific for *P. tattersalli* before

(Rumpler et al. 2004), are now considered as a common shift for all Indriidae [32] which has undergone an additional shift [33] in *P. tattersalli*. The common occurrence of *P. tattersalli* and *I. indri* was inferred in earlier studies. The FISH data also confirm the following human chromosome associations 3–21, 14–15, 12–22, which were already found with R-banding and to detect the following associations 7–16, 4–2, 8–15, 4–19, 6–4, but the last was not present in *Avahi*.

The comparison of R-banding patterns and FISH data allowed us to identify all autosomal euchromatic homologous chromosomes and chromosomal segments, and to define their presence/absence pattern in the analyzed species (Table 4).

The respective cladistic tree, based on presence/absence data (Supplementary Table S2), is depicted in Fig. 3. In this reconstruction, however, we allowed for hemiplasies (Avise and Robinson 2008; Robinson et al. 2008).

Accordingly, from *Microcebus* as a starting point, a common trunk bearing three centromere–telomere translocations [1,13] and one inversion [19] gives rise to two branches. One branch leads to *E. fulvus* which carries one Robertsonian translocation [2], one centromere–telomere translocation [26], and one centromere–telomere fission [9]. The second branch leads to the Indriidae. The Indriidae branch bears three shifts [14,30,32], one Robertsonian fission [20], and one inversion [43]. This branch then bifurcates into two clades. The first leading to *Avahi* carries three centromere–telomere translocations [16,17,29], four Robertsonian fissions [15,31,34,45], and one centromere–telomere fission [28]. Both *A. laniger* and *A. occidentalis* lineages contain each one non-homologous Robertsonian translocation [4 and 10] and *A. laniger* additionally a gain of heterochromatin [3].

The second branch bears four Robertsonian translocations [11,18,24,41] and two inversions [46,47]. The three rearrangements [11,18,41], common to *Propithecus*, are absent in *Indri*, and the rearrangement [22], common for *Indri* and *P. tattersalli*, but absent in *P. verreauxi*, can be regarded as hemiplasic. The respective branch diverges further into two subsequent lineages. The first is characterized by six Robertsonian translocations [6,21,25,36,39,51]. This lineage further diverges into a *P. edwardsi* and a *P. diadema* branch, which is defined by an additional

Table 3 Homologous chromosomal segments of human and Indriidae chromosomes based on R-banding techniques

HSA	MMR	EFU	ALA	AOC	PDI	PED	PVE	PTA	IIN
3 21	1[a]	1q ter[a]	1q ter[a]	1q ter[a]	1q ter[a]	1q ter	1q ter[a]	1q ter	1q[a]
1	2prox[a]	2p[a]	29[a]	32[a]	6p[a]	6p[a]	19[a]	13q[a]	9q ter[a]
1	2ter[a]	2q[a]	13[a]	12[a]	5q[a]	5q[a]	5q[a]	5q[a]	7q[a]
15 14	3[a]	3ter[a]	7+min prox[a]	6+min prox[a]	3q[a]	3q	9p+9q[a]	10p+10q	8p+8q[a]
4 2	4[a]	4[a]	4[a]	3[a]	2q[a]	2q	2q[a]	1p[a]	2q[a]
11	5[a]	6[a]	3q+min ter[a]	2q+min ter[a]	9 shift[a]	9 shift	8 shift[a]	9 shift	11 shift[a]
6	6	5[a]	5[a]	4[a]	2p[a]	2p	1p[a]	2q[a]	6q[a]
12 22	7	10[a]	26 14[a]	26 13[a]	10 shift[a]	10 shift[a]	10 shift[a]	11 shift[a]	12 shift[a]
2	8[a]	8[a]	11[a]	10[a]	4q[a]	4q	3q[a]	3q[a]	4p[a]
8 15	9[a]	7[a]	6[a]	5[a]	3p[a]	3p	3p[a]	3p	4q[a]
9	10	1p[a]	8	7	6q	6q	4p	4p	5p
7	11[a]	9[a]	9[a]	8[a]	1p[a]	1p[a]	2p[a]	2p[a]	2p[a]
5	12[a]	11[a]	10[a]	9[a]	4p	4p	4q[a]	4q[a]	5q[a]
13	13	12[a]	12[a]	11	7q	7q[a]	6q[a]	6q	3p[a]
6 4	14	13[a]	30 17[a]	30 16[a]	11 inv[a]	11 inv	11 inv[a]	8q prox+8p[a]	14 inv[a]
10		14[a]	min+min[a]	min+min	12 shift[a]	12 shift[a]	12 shift[a]	12 shift[a]	13 shift[a]
17	16	15[a]	15[a]	14[a]	8q[a]	8q[a]	7q[a]	7q[a]	3q inter[a]
1 7 16	17[a]	25 19[a]	2q[a]	1p[a]	8p[a]	8p[a]	7p[a]	7p[a]	6p[a]
20	18	17[a]	20[a]	19	5p[a]	5p	5p[a]	5p	7p[a]
18	19	16[a]	16[a]	17	15 inv[a]	15 inv	14 inv[a]	16 inv	15 inv[a]
16	20[a]	20[a]	2p[a]	24[a]	13q[a]	13q[a]	16[a]	8q ter[a]	10q ter[a]
22 12	21	24[a]	25[a]	25[a]	14q[a]	14q[a]	13q[a]	15q[a]	10q prox[a]
19	22[a]	3prox[a]	33[a]	33[a]	17 inv[a]	16 inv[a]	15 inv[a]	17 inv[a]	16 inv[a]
5	23[a]	21[a]	24[a]	23[a]	7p	7p	6p[a]	6p[a]	1p ter[a]
19	24[a]	18prox[a]	3p ter[a]	2p ter[a]	14p prox[a]	14p prox[a]	13p prox[a]	15p prox[a]	9q prox[a]
10	25[a]	22[a]	32[a]	29	16q[a]	18[a]	18[a]	14p[a]	3q ter[a]
8	26[a]	23[a]	23[a]	22	18	17[a]	17[a]	13p	9p[a]
1	27[a]	27[a]	28[a]	28[a]	13p[a]	13p[a]	21[a]	14q[a]	10p[a]
4	28	18ter[a]	3p prox	2p prox	14p ter	14p ter	13p ter	15p ter	9q inter
4	29	28[a]	27[a]	27[a]	16p[a]	19	20[a]	18[a]	17[a]
4	30	26[a]	31[a]	31[a]	19[a]	20	22[a]	19[a]	18[a]
3	31[a]	1q inter[a]	1q inter[a]	1q inter[a]	1q inter[a]	1q inter	1q inter[a]	1q inter	1p inter[a]
3	32[a]	1q prox[a]	1q prox[a]	1q prox[a]	1q prox[a]	1q prox	1q prox[a]	1q prox	1p prox[a]
		29	18	15	20	21	23	20	19
			19	18					
			21	20					
			22	21					
			34	34					

HSA Homo sapiens, MMR M. murinus, EFU E. fulvus, ALA A. laniger, AOC A. occidentalis, PDI P. diadema, PED P. edwardsi, PVE P. verreauxi, PTA P. tattersalli, IIN I. indri

[a] Validated by FISH

Table 4 List of associations identified in the Indriidae and outgroup taxa

1	//(32+31+1): EFU, ALA, AOC, PVE, PDI, PED, PTA, IIN
2	9//(32+31+1): EFU
3	h//(32+31+1): ALA
4	17//(32+31+1): AOC
5	6//(32+31+1): PVE
6	11//(32+31+1): PDI, PED
7	4//(32+31+1): PTA
8	23+(31+32)//1: IIN
9	//(17prox+17ter): ALA, AOC, PVE, PDI, PED, PTA, IIN
10	20//17: ALA
11	17//16: PVE, PDI, PED, PTA
12	17//6: IIN
13	//(24+28): EFU, ALA, AOC, PVE, PDI, PED, PTA, IIN
14	(5prox//5ter): PVE, PDI, PED, PTA, IIN
15	(5prox; 5ter): ALA, AOC
16	(24+28)//5ter: ALA, AOC
17	//(?+5prox): ALA, AOC
18	(28+24)//21: PVE, PDI, PED, PTA
19	(2prox//2ter): EFU
20	(2prox; 2ter): ALA, AOC
21	27//20: PDI, PED
22	26//2prox: PTA, IIN
23	26//(24+28)+2prox: IIN
24	18//2ter: PVE, PDI, PED, PTA, IIN
25	9//3: PDI, PED
26	//(22+3): EFU
27	(3prox//3ter): PVE, PTA, IIN
28	(3prox; 3ter): ALA, AOC
29	//(3prox+?): ALA, AOC
30	(7prox//7ter): PVE, PDI, PED, PTA, IIN
31	(7prox; 7ter): ALA, AOC
32	(15prox//15ter): PVE, PDI, PED, IIN
33	(15prox//15ter): PTA (another rearrangement)
34	(15prox; 15ter): ALA, AOC
35	11//4: PVE, IIN
36	6//4: PDI, PED
37	11//6: PTA
38	9//8: PVE, PTA, IIN
39	12//8: PDI, PED
40	12//10: PVE, PTA, IIN
41	23//13: PVE, PDI, PED, PTA
42	13//(?+16+25): IIN
43	(14prox//14ter): PVE, PDI, PED, PTA, IIN
44	(20+14prox)//14ter: PTA
45	(14prox; 14ter): ALA, AOC
46	(19prox//19ter): PVE, PDI, PED, PTA, IIN
47	(22prox//22ter): PVE, PDI, PED, PTA, IIN
48	29//25: PDI
49	25//27: PTA
50	27//(21+20): IIN
51	2prox//10: PDI, PED

"//" indicates centromeric position. ":" indicates a fission. "h" indicates heterochromatin. *Microcebus murinus* (MMR) chromosomes are the reference chromosomes. For other abbreviations, see Table 3

Robertsonian translocation [48]. The second branch, bearing one inversion [27] and three Robertsonian translocations [22,38,40], of which one [22] is hemiplasic, splits into a *P. verreauxi/Indri* branch with one additional Robertsonian translocation [35] and a *P. tattersalli* lineage with three Robertsonian translocations [7,37,49], one shift [33], and one centromere–telomere translocation [44]. The branch leading to *Indri* bears a Robertsonian translocation followed by one inversion [8], one Robertsonian translocation [12], one transposition [23], one Robertsonian translocation followed by two centromere–telomere translocations [42], and one Robertsonian translocation followed by a centromere–telomere translocation [50], while the branch leading to *P. verreauxi* carries one Robertsonian translocation [5].

In total, the 51 associations consist of 56 chromosomal rearrangements which comprise 26 Robertsonian translocations (the hemiplasic rearrangements have only be counted when they occurred on their polymorphic stage), six inversions, four shifts, 11 centromere–telomere translocations, five Robertsonian fissions, two centromere–telomere fissions, one transposition, and one gain of heterochromatin.

The reticulated phylogeny is depicted in Fig. 4. In principal, obtained relationships are the same as in Fig. 3, starting with the consecutive divergences of EFU and *Avahi*. The branch leading to *Indri* and *Propithecus* bears two inversions and one Robertsonian translocation [24,46,47]. The following reticulated evolution thus involves eight rearrangements, among them the four hemiplasic rearrangements [11,18,41,22]: two Robertsonian translocations [38,40] and one inversion [27] shared by *Indri*, *P. verreauxi*, and *P. tattersalli*; three Robertsonian translocations [11,18,41] shared by *P. verreauxi*, *P. tattersalli*, and *P. diadema*;
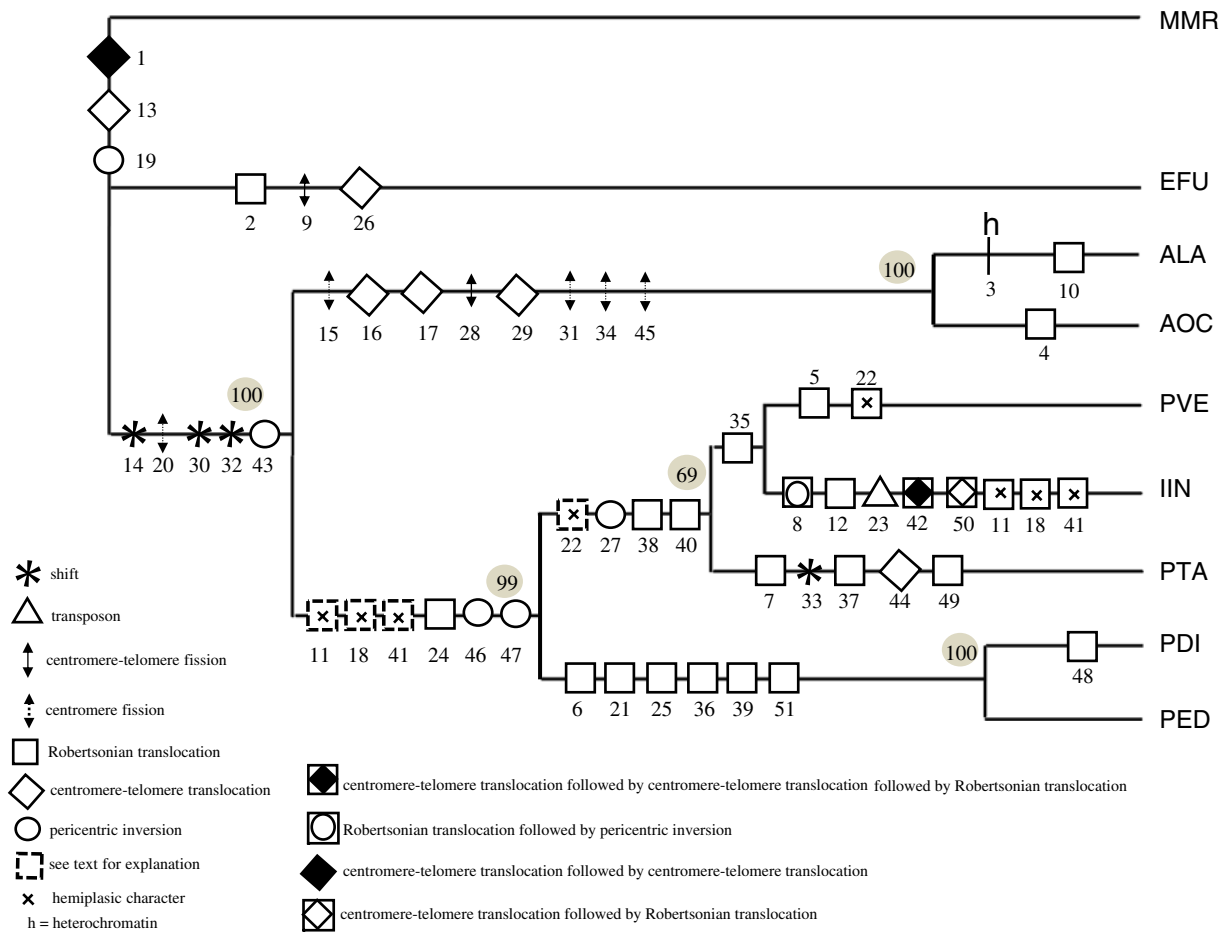
Fig. 3 Parsimony reconstruction of chromosomal changes allowing for hemiplasies. Numbering of rearrangements as in Table 4. Hemiplasic rearrangements are indicated by "X". "X" depicted in a *broken square* indicates an ancestral polymorphic stage of the respective rearrangement, whereas when depicted in *closed squares*, these rearrangements have not been retained in the lineage. *Numbers in gray circles* represent bootstrap values

one Robertsonian translocation shared by *Indri* and *P. verreauxi* [35]; and one Robertsonian translocation shared by *Indri* and *P. tattersalli* [22]. From this network, various branches emerge containing the respective specific rearrangements identical to those of the cladistic phylogeny. The branch leading to *Indri* bears nine rearrangements: one Robertsonian translocation followed by an inversion [8], one Robertsonian translocation [12], one transposition [23], one Robertsonian translocation followed by two centromere–telomere translocations [42], and one Robertsonian translocation followed by one centromere–telomere translocation [50]. The branch leading to *P. verreauxi* bears only one Robertsonian translocation [5], whereas the branch leading to *P. tattersalli* carries three Robertsonian translocations [7,37,49], one centromere–telomere translocation [44], and one shift [33]. The branch to *P. edwardsi* carries six Robertsonian translocations [6,21,25,36,39,51] and on the *P. diadema* lineage one additional Robertsonian translocation appears [48]. All 56 rearrangements were necessary to give rise to the tree without hemiplasy, convergence, or reversion.

Molecular genetics

Phylogenetic trees reconstructed on the basis of ML and Bayesian algorithms revealed identical tree topologies (Fig. 5). Although the monophyly of the Indriidae family was well supported, the relationships among the three genera *Avahi*, *Indri*, and *Propithecus* remained unresolved. Within *Avahi*, we observed a

Fig. 4 Reticulate phylogeny based on chromosomal rearrangements. Karyotype abbreviations as in Table 2. Numbers refer to chromosomal rearrangements as listed in Table 4. The gray oval depicts the reticulated phase, in which eight rearrangements are involved
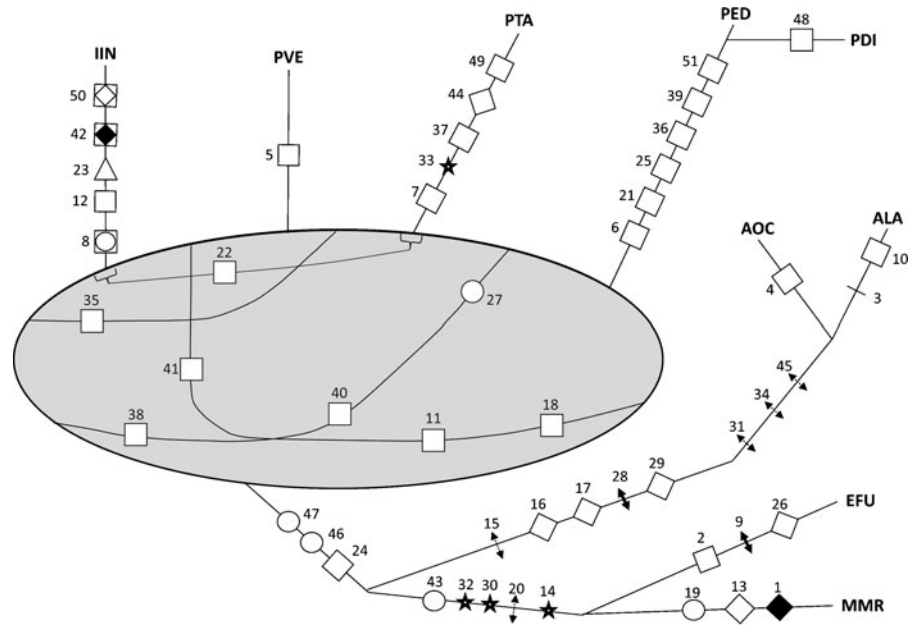
further division into an eastern clade containing *A. laniger*, *A. peyrierasi*, *A. meridionalis*, and *A. ramanantsoavani*, and a western clade with *A. cleesei* and *A. occidentalis*. The *Propithecus* clade further divides into two subgroups with geographically non-overlapping ranges. The first of these comprises the western populations of the *P. verreauxi* group and *P. tattersalli*, and the second the eastern populations of the *P. diadema* group. The latter further splits into four clades: *P. perrieri*, *P. diadema* 1, *P. diadema* 2, and *P. edwardsi*. *P. perrieri* occurs north of the eastern forest in Daraina, *P. diadema* 2 occurs in an area extending from Zahamena in the northern part till Maromizaha in the central eastern part of the *P. diadema* range, while *P. diadema* 1 encompasses the *P. diadema* 2 range in the North, West, and South (Fig. 1). The Mangoro/Onive Rivers comprise the southern limit of the *P. diadema* distribution. *P. edwardsi* contains all populations from the Mangoro/Onive Rivers in the north as far south as Anjamba and Ranomafana. The clade comprising members of the *P. verreauxi* group and *P. tattersalli* further diverges into two subclades. The southern subclade consists of *P. verreauxi*, *P. coronatus*, *P. deckeni*, and *P.* spp., and the northern subclade of *P. coquereli* and *P. tattersalli*, thus suggesting paraphyly of the *P. verreauxi* group. This appears particularly evident for *P. deckeni* and *P. coronatus* due to the morpho-

logical aspect of some animals (*P.* spp.) suggesting hybridization.

Discussion

Chromosomal evolution

In the course of the evolution of the Indriidae, the occurrence of several centromere–telomere translocations suggests that during chromosomal evolution new centromeres occurred while on some chromosomes, others were deactivated. These preserved their structural integrity in at least some chromosomes as a result of earlier centromere–telomere translocations. At the level of the obtained resolution, it is likely that the same breakpoints corresponding to active or latent centromeres were repeatedly used during the karyotype restructuring. This would also explain the occurrence of reverse mutations and probably the propensity to chromosomal polymorphisms (Kehrer-Sawatzki and Cooper 2008; Stanyon et al. 2008).

Comparison between cladistic and reticulated trees

In the cladistic tree, after the split between *Avahi* and the other Indriidae, four Robertsonian translocations [11,18,22,41] appeared as homoplasic. Indeed, three

Fig. 5 Phylogenetic
relationships among
Indriidae taxa based on
complete cyt *b* sequence
data. *Numbers on branches*
indicate support values as
obtained from Bayesian and
ML reconstructions,
respectively
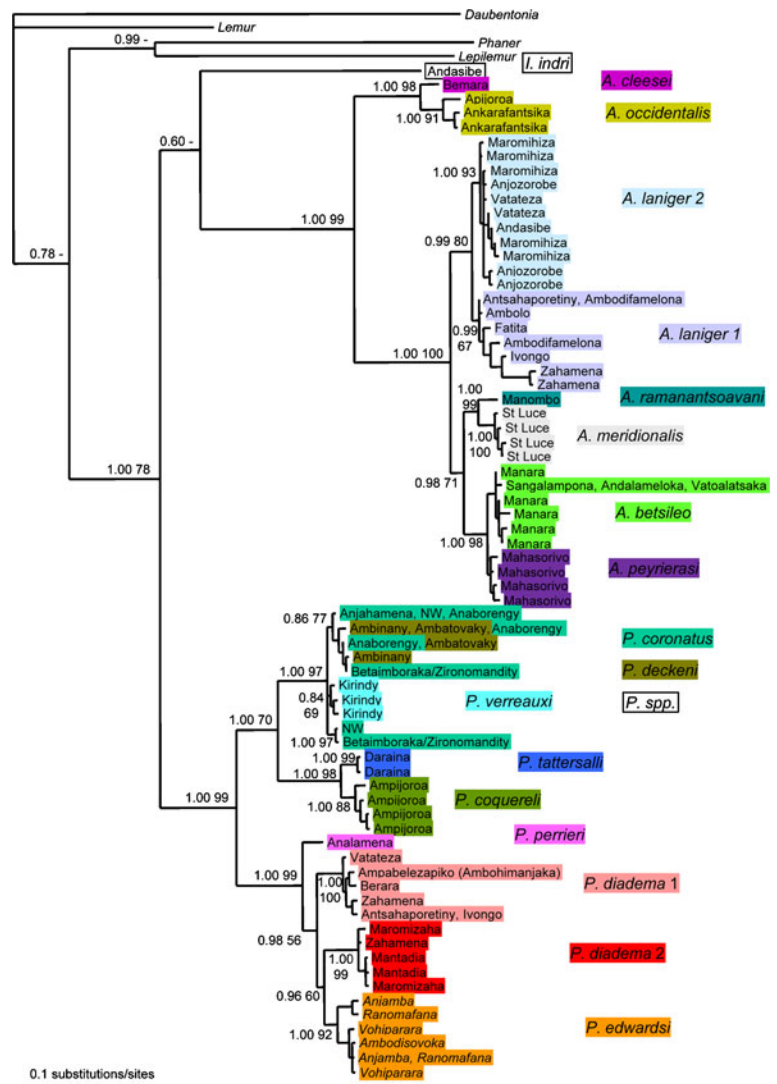
of them are present in all *Propithecus* but absent in
Indri and can be considered either as convergences, the
same rearrangements having occurred independently
on three branches, or as having undergone reversions
on the branch leading to *Indri*. The latter might be
most parsimonious. The fourth rearrangement, present
in *Indri* and *P. tattersalli* but absent in *P. verreauxi*, can
also be considered as the result of one convergence or
reversion. By using the interpretative hypothesis of
Avise and Robinson (2008) and Robinson et al. (2008)
termed hemiplasy, these four rearrangements are
considered as having been ancestrally present on a
polymorphic stage and they were only retained in some
karyotypes while they disappeared in others. Hemi-
plasy is more plausible for neutral polymorphism such

as Robertsonian translocations which have little or no
impact on fertility (Robinson et al. 2008). Indeed,
crosses between different species or subspecies of
lemurs differing by six and eight rearrangements have
been observed in the wild and in captivity, respectively,
without reducing the viability of the hybrids (Rumpler
and Dutrillaux 1990). A reduction of fertility in male
hybrids is nevertheless observed if the Robertsonian
translocations involve the same acrocentric chromo-
somes but in different combinations, so that the
resulting metacentric chromosomes show monobrachial
homologies. This leads to the formation of meiotic
multivalents and to significant gametogenic impairment
if the multivalents comprise more than four chromo-
somes. In females, in contrast, ovogenesis seems not to

be disturbed in species studied so far (Rumpler and Dutrillaux 1990). In the chromosomal evolution of the Indriidae, the Robertsonian translocations involved in the polymorphic stage would give rise to multiple independent heterozygosities and none multivalents, and hence, would be no major obstacle for a successful reproduction. Conversely, on the specific branches, the Robertsonian translocations gave rise to chromosomes showing monobrachial homologies which are more selective in terms of speciation.

In the reticulated evolution, eight rearrangements (seven Robertsonian translocations and one inversion) are involved in the reticulated process. Among them the four abovementioned Robertsonian translocations [11,18,22,41] and three [27,38,40] occurring on the common branch to *P. verreauxi/P. tattersalli/Indri* and one [35] on the branch to *P. verreauxi* and *I. indri*. Hybridization during this phase would give rise to seven independent trivalents and a single inversion loop, which would not necessarily reduce fertility of the heterozygotes. Conversely, the rearrangements on the taxon-specific branches to *Indri*, *P. verreauxi*, *P. tattersalli*, and *P. edwardsi/P. diadema*, which are identical in both phylogenies, will have the same speciation effect. It appears to us that the cladistic phylogeny with four polymorphic (hemiplasic) rearrangements is the most likely and the most similar to the molecular phylogeny. Thus, we will retain it for the comparison between chromosomal and molecular phylogenies.

Chromosomal versus molecular phylogeny

By comparing phylogenetic trees inferred from chromosomal (Fig. 3) and molecular data (Fig. 5), some identical relationships among species and clades became obvious. In both phylogenies, the *Avahi* clade as well as the sister group relationships of *P. diadema/P. edwardsi* to *P. verreauxi/P. tattersalli* are strongly supported. Moreover, a major split within the genus *Avahi* separating the eastern and the western species is also strongly supported by both phylogenies even if the chromosome characteristics do not allow distinguish between as many species as the molecular data.

However, the following relationships differ among phylogenies: (1) Although phylogenetic relationships among the three Indriidae genera are not resolved, the molecular phylogeny indicates an early emergence of *Indri* while in the chromosomal phylogenies *Indri* is

nested within *Propithecus*. (2) Whereas the chromosome data indicate a clade consisting of *P. coquereli*, *P. deckeni*, *P. coronatus*, and *P. verreauxi*, the mitochondrial phylogeny suggests a closer relationship of *P. coquereli* to *P. tattersalli* than to the other taxa of the *P. verreauxi* group. (3) Overall, many more species are identified when using molecular patterns than when characterizing species in terms of their karyotype. This is particularly obvious for *Avahi* where chromosome changes only allow to distinguish between an eastern and a western taxon, whereas the molecular data identifies five species within the eastern group and two species within the western one (Andriantompohavana et al. 2007; Zaramody et al. 2006). Similarly, among *Propithecus*, *P. verreauxi/P. coronatus/P. deckeni* and *P. coquereli* share the same karyotype, but the molecular data clearly distinguish *P. coquereli* as a separate taxon, which constitutes the sister taxon to *P. tattersalli*. The other three taxa of this group form a distinct clade but further taxonomic differentiation is not possible based on cyt *b* sequence variation. *P. verreauxi*, *P. coronatus*, and *P. deckeni* as well as morphological hybrid forms between *P. coronatus* and *P. deckeni* cluster in the same clade, thus providing no arguments for a classification into distinct species (Pastorini et al. 2001, but see Thalmann et al. 2002 for distinction into subspecies).

Among the eastern *Propithecus*, *P. edwardsi* has a specific karyotype, separating it from the other eastern *Propithecus* taxa, which all share the same karyotype (*P. diadema*, *P. perrieri*, *P. candidus*). The molecular data, however, suggest a possible specific status for *P. diadema*, *P. candidus*, and *P. perrieri* besides *P. edwardsi* (Mayor et al. 2004).

Our molecular analysis revealed two well-supported mitochondrial clades in *P. diadema*. One (*P. diadema* 2) is most likely closer related to *P. edwardsi* than to its conspecific clade (*P. diadema* 1). Taking only the genetic distance into account, one would have to split *P. diadema* into two taxa because the genetic distance is at least as large as for *P. tattersalli* and *P. coquereli*. Morphologically, however, no clade-specific differences are known karyotypically; both clades are the same and evidence from nuclear genetic markers is not yet available. Therefore, we think that the current data do not warrant the delineation of a new taxon. Instead, we propose male introgression as a possible cause for the paraphyly of *P. diadema* (Funk and Omland 2003). We envision three isolated ances-

tral *Propithecus* populations along the central and east coast of Madagascar. The southern population refers to proto-*P. edwardsi*, the central to proto-*P. diadema* 2, and the northern to proto-*P. diadema* 1. After a phase of isolation, where population-specific mitochondrial clades evolved, the northern and central populations came into secondary contact. Males of the northern population invaded the central population and reproduced successfully. Hybrid females backcrossed with new invading northern males over generations. As a result, the central population would be swamped with northern nuclear DNA, but would retain their own mitochondria, which was more closely related to that from *P. edwardsi* than to the mitochondria from the northern population. Today, morphology and nuclear genome of the introgressed central population is very similar or identical to the northern population *P. diadema*, but its mitochondria are still the original and closely related to *P. edwardsi*. At one site, Zahamena, individuals of both *P. diadema* haplogroups are found sympatrically.

Natural hybridization and introgression have recently been recognized as one evolutionary mechanism of speciation for many vertebrate species (Seehausen 2004; Mallet 2005) including primates (review in Arnold and Meyer 2006). However, hybridization between species does not necessarily lead to the formation of new species. In many cases, hybrid offspring suffer from reduced viability or they are sterile. Sex-specific introgressive hybridization is of particular interest because here the result can be the capture of mitochondria or Y chromosomes followed or not followed by nuclear swamping. The genome of the introgressed population or species changes due to horizontal gene transfer without speciation (Zinner et al. 2009a, b).

Indeed, lemurs often hybridize in the wild resulting in horizontal gene transfer (Fausser et al. 2002; Wyner et al. 2002; Pastorini et al. 2009). Therefore, a dichotomic phylogeny might not necessarily indicate a complete interruption of gene flow between different clades, and this becomes particularly relevant when the bootstrap values are very low as it is the case for the topology of *Indri*. Reticulate evolution was also hypothesized on the chromosomal level, suggesting that in a number of species the chromosomal evolution passed through phases of reticulation, during which chromosome rearrangements could be exchanged among populations of diverging lineages, and cladogenesis, when molecular mutations and/or chromo-

somal changes have a stronger selective effect (Dutrillaux 1986; Moulin et al. 2008).

We propose a synthetic scheme in which both kinds of events, chromosomal and molecular ones, played a major role during the Indriidae speciation process.

Among *Avahi*, a chromosomal and a molecular barrier between the eastern and western populations were followed by molecular changes giving rise to numerous species without any chromosomal changes. Among *Propithecus*, hemiplasic chromosomal changes occurred (Fig. 3) while the molecular evolution split this group into several subgroups or proto-taxa. These ancestral subgroups probably were not yet reproductively isolated and the reproductive barriers appeared later with, or due to, the specific chromosome changes. Proto-*P. edwardsi* differentiated into a species characterized by chromosomal and molecular changes, while *P. diadema* experienced only molecular changes which allowed distinguishing at least subspecies: *P. d. diadema*, *P. d. perrieri*, and *P. d. candidus*. Among the *P. coronatus*, *P. verreauxi*, *P. deckeni/P. coquereli*, and *P. tattersalli* ancestral populations, molecular changes occurred south of the Betsiboka River and gene flow appeared to be maintained between *P. verreauxi*, *P. coronatus*, and *P. deckeni* while north of this river molecular evolution first isolated *P. coquereli* and *P. tattersalli* from the *P. verreauxi*, *P. coronatus*, and *P. deckeni* group and later *P. coquereli* from *P. tattersalli*. The latter accumulated numerous chromosome changes. We cannot specify precisely if these were at the origin of its divergence from *P. coquereli*.

Both chromosome and molecular phylogenies are in accordance with the general biogeographical divisions for Madagascar (Martin 2000). The distributions of *Propithecus* and *Avahi* follow the traditionally recognized east–west and north–south zoogeographical divisions (Martin 2000; Wilmé et al. 2006; Thalmann 2007; Rumpler et al. 2008). Both mitochondrial and chromosomal phylogenies are consistent with a primary east/west division between *Avahi* and *Propithecus/Indri*. Within the eastern region, the Mangoro–Onive River system constituted a major geographical barrier separating the northern from the southern forms. Within the western region, the Betsiboka River also constitutes a major geographical barrier separating northern from southern taxa. The grouping of *P. tattersalli* and *P. coquereli* also indicates ancestral transverse relationships between the species living in the eastern and

western domain similar to that observed between *Lepilemur edwardsi* and *Lepilemur microdon* (Andriaholinirina et al. 2006).

Conclusion

Our chromosomal and molecular analyses of Indriidae are complementary approaches to propose a unified model of the evolutionary history of this lemur family which corresponds to their current zoogeographical distribution. Both approaches show that *Avahi* diverged in an eastern and western group followed by differentiation on the mitochondrial level but without any chromosomal changes. For the *Propithecus*/*Indri* clade, the cladistic chromosomal phylogeny has been retained as the most likely with four hemiplasic rearrangements for which an ancestral polymorphism has to be assumed. Both cytogenetic and molecular phylogenies show a clear distinction between the western (*P. verreauxi*, *P. coquereli*, and *P. tattersalli*) and eastern domain species (*P. diadema* and *P. edwardsi*). Neither chromosomal nor molecular data, however, support a separation of the three western *Propithecus* forms, *P. verreauxi*, *P. coronatus*, and *P. deckeni*, into distinct species. On the other side, both datasets suggest the Mangoro–Onive Rivers as the barrier between *P. edwardsi* and *P. diadema* in the eastern Malagasy forests.

References

Andriaholinirina N, Fausser J-L, Roos C et al (2006) Molecular phylogeny and taxonomic revision of the sportive lemurs (*Lepilemur*, Primates). BMC Evol Biol 6:17

Andriantompohavana R, Lei R, Zaonarivelo JR et al (2007) Molecular phylogeny and taxonomic revision of the woolly lemurs, genus *Avahi* (Primates: Lemuriformes). Texas Tech Univ Special Publ 51:1–59

Arnold ML, Meyer A (2006) Natural hybridization in primates: one evolutionary mechanism. Zoology 109:261–276

Avise JC, Robinson T (2008) Hemiplasy: a new term in the lexicon of phylogenetics. Syst Biol 57:503–507

Britton-Davidian J, Catalan J, Da Graça RM et al (2005) Chromosomal phylogeny of Robertsonian races of the house mouse on the island of Madeira: testing between alternative mutational processes. Genet Res 86:171–183

Curtis DJ, Velo A, Raheliarisoa EO, Zaramody A, Mueller P (1998) Surveys on *Propithecus verreauxi deckeni*, a melanistic variant, and *P. v. coronatus* in north-west Madagascar. Oryx 32:157–164

Delpero M, Masters JC, Cervella P, Crovella S, Ardito G, Rumpler Y (2001) Phylogenetic relationships among the Malagasy lemuriforms (Primates: Strepsirrhini) as indicated by mitochondrial sequence data from the 12S rRNA gene. Zool J Linn Soc 133:83–103

Dobigny G, Ducroz JF, Robinson TJ, Volobouev V (2004) Cytogenetics and cladistics. Syst Biol 53:470–484

Dutrillaux B (1986) Relations chromosomiques entre sous-ordres et infra-ordres, et schéma évolutif général des Primates. In: Evolution chromosomique chez les Primates, les Carnivores et les Rongeurs. Mammalia 50:108–121

Fausser JL, Prosper P, Donati G, Ramanamanjato JB, Rumpler Y (2002) Phylogenetic relationships between *Hapalemur* species and subspecies based on mitochondrial DNA sequences. BMC Evol Biol 2:4

Funk D, Omland K (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Ann Rev Ecol Evol Syst 34:397–423

Hill WCO (1953) Primates: comparative anatomy and taxonomy, vol. I. Strepsirrhines. University Press, Edinburgh

Horvath JE, Weisrock DW, Embry SL et al (2008) Development and application of a phylogenomic toolkit: resolving the evolutionary history of Madagascar's lemurs. Genome Res 18:489–499

Kehrer-Sawatzki H, Cooper DN (2008) Molecular mechanisms of chromosomal rearrangement during primate evolution. Chromosome Res 16:41–56

Lei R, Engberg SE, Andriantompohavana R et al (2008) Nocturnal lemur diversity at Masoala National Park. Texas Tech Univ Special Publ 53:1–41

Mallet J (2005) Hybridization as an invasion of the genome. Trends Ecol Evol 20:229–237

Martin RD (2000) Origins, diversity and relationships of lemurs. Int J Primatol 21:1021–1049

Mayor MI, Sommer JA, Houck ML et al (2004) Specific status of *Propithecus* spp. Int J Primatol 25:875–900

Mittermeier RA, Louis EE Jr, Richardson M et al (2010) Lemurs of Madagascar, 3rd edn. Conservation International, Arlington

Moulin S, Gerbault-Seureau M, Dutrillaux B, Richard F (2008) Phylogenomics of African guenons. Chromosome Res 16:783–799

Pastorini J, Forstner MRJ, Martin RD (2001) Phylogenetic history of sifakas (Propithecus: Lemuriformes) derived from mtDNA sequences. Am J Primatol 53:1–17

Petter JJ, Albignac R, Rumpler Y (1977) Mammifères lémuriens (Primates prosimiens). In: Faune de Madagascar, vol. 44. CNRS/ORSTOM, Paris, pp 1–512

Posada B, Crandall KA (1998) MODELTEST: testing the model of DNA substitutions. Bioinformatics 14:817–818

Powzyk J, Thalmann U (2003) Indri indri, Indri. In: Goodman SM, Benstead JP (eds) The natural history of Madagascar. University of Chicago Press, Chicago, pp 1342–1345

Qumsiyeh MB, Hamilton MJ, Schlitter DA (1987) Problems in using Robertsonian rearrangements in determining monophyly: examples from the genera Tatera and Gerbillurus. Cytogenet Cell Genet 44:198–208

Robinson TJ, Ruiz-Herrera A, Avise JC (2008) Hemiplasy and homoplasy in the karyotypic phylogenies of mammals. Proc Natl Acad Sci USA 105:14477–14481

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Roos C, Schmitz J, Zischler H (2004) Primate jumping genes elucidate strepsirrhine phylogeny. Proc Natl Acad Sci USA 101:10650–10654

Rumpler Y, Albignac R (1979) Cytogenetic of the Indriidae Bennet, 1832, an endemic Malagasy lemur family. In: Chiarelli B, Koen AL, Ardito G (eds) Comparative karyology of primates. Mouton, New York, pp 29–34

Rumpler Y, Dutrillaux B (1990) Chromosomal evolution and speciation in primates. In: RBC, University of the Basque Country (ed) Volume 23, review. Springer, Berlin, pp 1–112

Rumpler Y, Warter S, Ishak B, Dutrillaux B (1988) Chromosomal evolution in Malagasy lemurs: X. Chromosomal banding studies of Propithecus diadema edwardsi and Indri indri and phylogenic relationships between all the species of the Indriidae. Am J Primatol 16:63–71

Rumpler Y, Warter S, Rabarivola C, Petter JJ, Dutrillaux B (1990) Chromosomal evolution in Malagasy lemurs: XII. Chromosomal banding study of Avahi laniger occidentalis (Syn: Lichanotus laniger occidentalis) and cytogenetic data in favour of its classification in a species apart—Avahi occidentalis. Am J Primatol 21:307–316

Rumpler Y, Andriaholinirina N, Warter S, Hauwy M, Rabarivola C (2004) Phylogenetic history of the sifakas (Propithecus: Lemuriformes) derived from cytogenetic studies. Chromosome Res 12:453–463

Rumpler Y, Warter S, Hauwy M, Jean-Luc Fausser JL, Roos C, Zinner D (2008) Comparing chromosomal and mitochondrial phylogenies of sportive lemurs (Genus Lepilemur, Primates). Chromosome Res 16:1075–1250

Schwarz E (1931) A revision of the genera and species of Madagascar Lemuridae. Proc Zool Soc London 1931:399–428

Seehausen O (2004) Hybridization and adaptive radiation. Trends Ecol Evol 19:198–207

Simons EL (1988) A new species of Propithecus (Primates) from Northeast Madagascar. Folia Primatol 50:143–151

Stanyon R, Rocchi M, Capozzi O et al (2008) Primate chromosome evolution: ancestral karyotypes, marker order and neocentromeres. Chromosome Res 16:17–39

Swofford D (2000) PAUP*4.0b.4a. Phylogenetic analysis using parsimony* (and other methods). Sinauer, Sunderland

Tattersall I (1982) The primates of Madagascar. Columbia University Press, New York

Tattersall I (1986) Notes on the distribution and taxonomic status of some subspecies of Propithecus in Madagascar. Folia Primatol 46:51–63

Thalmann U (2007) Biodiversity, phylogeography, biogeography and conservation: lemurs as an example. Folia Primatol 78:420–443

Thalmann U, Geissmann T (2000) Distribution and geographic variation in the western woolly lemur (Avahi ocidentalis) with description of a new species (A. unicolor). Int J Primatol 21:915–941

Thalmann U, Geissmann T (2005) New species of woolly lemur Avahi (Primates: Lemoriformes) in Bemaraha (central Western Madagascar). Am J Primatol 67:371–376

Thalmann U, Kümmerli R, Zaramody A (2002) Why Propithecus verreauxi deckeni and P. v. coronatus are valid taxa—quantitative and qualitative arguments. Lemur News 7:11–16

Vezuli A, Rumpler Y (2000) Lemur chromosomal study with simultaneous R-banding and chromosome painting. Chromosoma 109:214–218

Volobouev VT, AniskinVM LE, Ducroz JF (2002) Patterns of karyotype evolution in complexes of sibling species within three genera of African murid rodents inferred from the comparison of cytogenetic and molecular data. Cytogenet Genome Res 96:261–275

Warter S, Hauwy M, Dutrillaux B, Rumpler Y (2005) Application of molecular cytogenetics for chromosomal evolution of the Lemuriformes (Prosimians). Cytogenet Genome Res 108:197–203

Wilmé L, Goodman SM, Ganzhorn JU (2006) Biogeographic evolution of Madagascar's microendemic biota. Science 312:1063

Wyner YM, Johnson SE, Stumpf RM, DeSalle R (2002) Genetic assessment of a white-collared x red-fronted lemur hybrid zone at Andringitra, Madagascar. Am J Primatol 67:51–66

Zaramody A, Fausser J, Roos C et al (2006) Molecular phylogeny and taxonomic revision of the eastern woolly lemurs (Avahi laniger). Primate Report 74:9–23

Zinner D, Groeneveld LF, Keller C, Roos C (2009a) Mitochondrial phylogeography of baboons (Papio spp.)—indication for introgressive hybridization? BMC Evol Biol 9:83

Zinner D, Arnold ML, Roos C (2009b) Is the new primate genus Rungwecebus a baboon? PLoS ONE 4:e4859

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence data sets under the maximum likelihood criterion. Ph.D. thesis, The University of Texas at Austin