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Karyotypic Fission Theory Applied Kinetochore Reproduction and Lemur Evolution

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Abstract

Karyological analyses of lemur taxa reveal Robertsonian chromosome rearrangements and pericentric inversions. Karyotypic fission (kinetochore reproduction) theory potentially explains lemur karyotype evolution. Application of the theory suggests that modern lemurs are derived from an ancestral primate with a diploid number of 20. All extant karyotypes of lepilemurid, lemurid, daubentoniid, indrid, and cheirogaleid are most parsimoniously explained as the product of four karyotypic fissions, two primary and two secondary, followed by pericentric inversions. The first fissioning event generated the karyotypic diversity that later became fixed in the ancestral stocks of the Daubentoniidae, Indridae, and a common lemurid-cheirogaleid stock. The ancestral stock of the Lepilemuridae was not affected by this event, but did experience a later independent fissioning of the ancestral 2N=20, generating modern diploid numbers ranging from 20–38. A secondary fissioning event isolated the Lemuridae and Cheirogaleidae from one another. A separate secondary fissioning explains indrid karyotypes. Kinetochore research supplies mechanisms for karyotypic fissioning followed by normal segregation of fission-generated acrocentric chromosomes. This analysis is consistent with the theory of the origins of eukaryotic cells, including the mitotic and meiotic motility system, from fusion of archaebacterial and eubacterial (spirochete) lineages. *Ad hoc* theories of Robertsonian fusions require approximately 100 independent events to generate the karyotypes of lemurs. Fission theory, consistent with the tendency of former endosymbionts to reproduce at different rates from their host cells, requires only 2–4 independent events.

Keywords: Lepilemuridae, Lemuridae, Daubentoniidae, Indridae, Cheirogaleidae, Robertsonian rearrangements, evolutionary cytogenetics, pericentric inversion, chromosomal evolution, kinetochore, centromere, KFE (Karyotypic fissioning event)

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1. Introduction

The symbiogenetic acquisition of microtubule organizing centers (MTOCs) and microtubule-based organelles including undulipodia (eukaryotic "flagella"), from spirochete association with an archaebacterium, is claimed to be the first step in eukaryosis, the evolutionary force that led to the first eukaryotic cells from prokaryotes (Margulis, 1996). The evolutionary lineage of microtubules is supported by the similarity of alpha and beta tubulin protein in mammalian cells and those associated in protists (Margulis, 1993). The evolutionary conservation of centromeric DNA, proposed to code for proteins involved in the function and/or structure of the eukaryotic kinetochore, has been demonstrated by comparative sequencing of DNA from humans, protists, metazoans, and plants (Brown, 1995; Lapenta et al., 1997). The autonomous nature of kinetochores, discussed here, provides further support for the Serial Endosymbiosis Theory; that mitotic spindle and MTOCs, essential prerequisites for meiosis, arose through symbiogenesis (Margulis, 1993). Kinetochores are essential for spindle formation and chromosomal segregation during any eukaryotic cell division. Analysis of lemur karyotypes suggests that kinetochore behavior mediated the diversification of their chromosomal arrangements (i.e., their karyotypes) which likely led to genetic incompatibility amongst species.

Chromosomal complements of over 1,000 mammalian species are now known and diploid numbers are found to range between 2N=6 and 92 (Hsu and Benirschke, 1967–1975; Matthey, 1973). Karyotype analysis is useful for drawing inferences about phylogenetic relationships among species. Three separate theories have been put forth to explain chromosomal evolution leading to mammalian karyotype diversity. Fusion theory postulates an ancestral mammalian diploid number of 80 to 96 in animals with all acrocentric chromosomes (Ohno, 1969). Karyotypic fission theory, by contrast, postulates an ancestral mammalian diploid number of 14 mediocentric chromosomes (Todd, 1967) and is consistent with the fact that the didelphid marsupials, considered to be the most primitive true mammals, have diploid numbers ranging from 14 to 22 (Reig and Bianchi, 1969). Modal theory suggests an ancestral mammalian diploid number between 40 and 56 that generates karyotypes with higher and lower diploid numbers through both fissioning and fusion (Matthey, 1973). Imai (1978), who evaluated theories of mammalian karyotypic evolution (fusion, fission, or modal) by examining 723 mammalian karyotypes, supported fissioning as the most probable of the three mechanisms. Approximately one third of mammalian chromosomes are telocentric. Unlike the fusion idea, the karyotypic fission theory easily explains the origin of mammalian telocentric chromosomes.

The conservation of the ancestral mediocentric X chromosome in mammals

appears to have been favored by selection during eutherian mammalian chromosomal evolution. In such mammals, the X chromosome universally comprises approximately 5% of the genome (Ohno et al., 1964). The inheritance of fissioned sex chromosomes is apparently deleterious where partial aneuploidy is likely to be generated or transmitted at gametogenesis. Although fissioned autosomal homologues pair without difficulty, proper pairing of fissioned X and Y chromosomes may be inhibited by limited sex chromosome synapsis (at a small region of chromatin). A fission-generated acrocentric sex chromosome contains too few homologous base pairs to synapse with its mediocentric homologue, which tends to lead to failure of proper segregation. Inheritance of ancestral nonfissioned X chromosomes and/or acrocentric X chromosomes resulting from inversion theoretically yields viable offspring. Because non-fissioned ancestral sex chromosomes tend to be selectively retained, fissioning events tend to maximally increase chromosome number to two times the original diploid value minus two (Todd, 1992).

2. Karyotypic Fissioning and the Evolution of Lemurs

Lemurs are strepsirrhine primates indigenous only to the island of Madagascar. Lemuriformes comprise eight families of which five (Indridae, Lepilemuridae, Lemuridae, Daubentoniidae, and Cheirogaleidae) are extant (Jenkins, 1987). Living lemurs represent 36% of all primate families. Here I apply karyotypic fission theory to explain chromosome numbers and polymorphism in lemur karyotypes and show its usefulness in the reconstruction of lemur evolution.

There are 32 living species of lemurs and their diploid numbers range from 20 to 70 (Table 1). The karyotype of *Lepilemur ruficaudatus* approximates the ancestral arrangement for all lemurs postulated by application of karyotypic fission theory for it has the lowest diploid number found in lemurs and it is comprised of large mediocentric chromosomes. *Avahi occidentalis* has the highest lemurid diploid number and its karyotype is comprised of small, mostly acrocentric chromosomes and is therefore considered derived. The largest chromosome in the karyotype of *Avahi occidentalis* is approximately two thirds the size of the largest in *Lepilemur ruficaudatus*.

Analysis of lemur karyotype morphology and available DNA banding studies indicate that lemur karyotypic diversification occurred mainly through Robertsonian rearrangements (i.e., fission or fusion of chromosomes) and pericentric inversions (Dutrillaux and Rumpler, 1977; Rumpler and Dutrillaux, 1976, 1978, 1979, 1980; Rumpler et al., 1983, 1985, 1986, 1988, 1990, 1991). Although Robertsonian rearrangements explain the range of diploid numbers found in lemurs, the paradox of polarity (i.e., the directionality of chromo-

Table 1. Lemur karyotype references

Species	2N	M	A	XY	HA	References
Lepilemur ruficaudatus	20	18	0	MA	20	Rumpler et al. (1985), Rumpler, Albignac (1977)
Lepilemur edwardsi	22	18	2	AA	20	Rumpler et al. (1986), Rumpler, Albignac (1977)
Lepilemur mustelinus	34	6	26	SA	20	Rumpler et al. (1986), Rumpler, Albignac (1977)
Lepilemur septentrionalis septentrionalis	34	6	26	MA	20	Rumpler et al. (1985), Rumpler, Albignac (1977)
L. s. ankaranensis	36	4	30	MA	20	Rumpler et al. (1985), Rumpler, Albignac (1977)
L. s. sahafarensis	36	4	30	MA	20	Rumpler, Albignac (1977)
L. s. andrafiamenensis	38	2	34	MA	20	Rumpler, Albignac (1977)
Lepilemur dorsalis	26	20	4	MA	20	Rumpler et al. (1986)
Lepilemur leucopus	26	20	4	MA	20	Rumpler et al. (1985)
Daubentonia madagascariensis	30	24	4	MA	20	Tagle et al. (1990), Rumpler (1975)
Eulemur macaco	44	20	22	AA	34	Rumpler, Dutrillaux (1976), Wurster-Hill (1973), Egozcue (1967), Chu, Bender (1961), Chu, Smomley (1961)
Eulemur coronatus	46	18	26	AA	34	Rumpler, Dutrillaux (1979)
Eulemur fulvus fulvus	48	16	30	AA	34	Chu, Smomley (1961)
	60	4	54	AA	34	Rumpler, Dutrillaux (1990), Rumpler, Dutrillaux (1976)
E. f. albocollaris	48	16	30	AA	34	Rumpler, Dutrillaux (1976)
E. f. collaris	52	12	38	AA	34	Rumpler, Dutrillaux (1976)
E. f. rufus	60	4	54	AA	34	Chu, Swomley (1961)
E. f. albifrons	60	4	54	AA	34	Rumpler, Dutrillaux (1980), Chu, Swomley (1961)
E. f. sanfordi	60	4	54	AA	34	Rumpler (1975)
Eulemur mongoz	60	4	54	AA	34	Rumpler, Dutrillaux (1990), Rumpler, Dutrillaux (1976), Chu, Swomley (1961)
Eulemur rubriventer	50	14	34	AA	34	Rumpler, Dutrillaux (1980)
Hapalemur aureus	62	0	60	AA	34	Rumpler et al. (1991)
Hapalemur simus	60	4	54	SA	34	Rumpler, Dutrillaux (1978)
Hapalemur griseus	54	10	42	AA	34	Rumpler, Albignac (1973), Chu, Swomley (1961)
		8	44	AA	34	Chu (1975)
H. g. alaotrensis	54	10	42	AA	34	Rumpler (1975)
H. g. meridionalis	54	10	42	AA	34	Warter et al. (1987)
H. g. spp.	56	8	46	AA	34	Rumpler, Dutrillaux (1978)
H. g. occidentalis	58	6	50	AA	34	Rumpler, Dutrillaux (1978), Rumpler, Albignac (1973)

Table 1. Lemur karyotype references. Continuation

Species	2N	M	A	XY	HA	References
H. g. olivareus	58	6	50	AA	34	Chu (1975), Chu, Swomley (1961)
Lemur catta	56	8	46	MA	34	Rumpler, Dutrillaux (1978), Rumpler (1970)
Varecia variegata	46	18	26	SA	34	Rumpler, Dutrillaux (1979), Rumpler (1970)
Microcebus murinus murinus	66	0	64	MA	34	Rumpler, Dutrillaux (1976), Rumpler, Albignac (1973)
M. m. rufus	66	0	64	MA	34	Rumpler, Albignac (1973)
Microcebus coquereli	66	0	64	SA	34	Rumpler, Dutrillaux (1979)
Allocebus trichotis	66	0	64	MA	34	Rumpler et al. (1995)
Cheirogaleus major	66	2	62	MA	34	Wurster-Hill (1973)
		0	64	SA	34	Rumpler, Dutrillaux (1979)
Cheirogaleus medius	66	2	62	MA	34	Wurster-Hill (1973)
		0	64	MA	34	Rumpler, Albignac (1973)
Phaner furcifer	46	16	28	MA	34	Rumpler, Dutrillaux (1979), Rumpler, Albignac (1973)
Avahi occidentalis	70	4	64	SA	38	Rumpler et al. (1990)
Avahi laniger	70	6	62	SA	38	Rumpler et al (1983)
Indri indri	40	30	8	SA	38	Rumpler et al. (1988)
Propithecus diadema edwardsi	44	32	10	SA	38	Rumpler et al. (1988)

M = Mediocentric (metacentric, and submetacentric chromosomes), A = Acrocentric, S = Submetacentric, HA = Hypothetical ancestral diploid number.

somal evolution) must be addressed. The current view of lemur chromosome evolution is that a high ancestral diploid number successively led to lower diploid numbers by several independent sequential mutations where each centric fusion reduced the chromosome number by two (in homozygotic offspring). Generating a range of diploid numbers from 20 to 70 via sequential fusion, fission, or both necessitates the persistence of numerous chromosomal mutations, each an unlikely event. Rumpler (1990) postulates 101 independent Robertsonian rearrangements to explain lemur karyotypes. Karyotypic fissioning increases the likelihood of establishing a range of diploid numbers in that a single event may potentially introduce a full complement of fissioned autosomes into a population. A diploid range from 20 to 70 chromosomes can be explained by only two karyotypic fissioning events (Fig. 1).

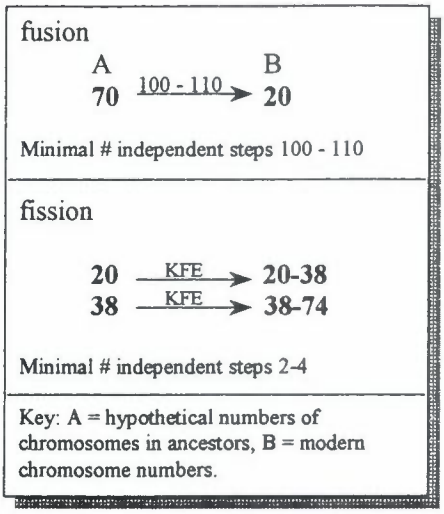

Figure 1. Fusion and fission theory compared for lemur evolution. The current view of lemur chromosomal evolution favors fusion; it assumes high ancestral chromosome numbers and postulates that 101 independent Robertsonian rearrangements have occurred (top). Generation of a range of diploid numbers, from 20 to 70, via sequential fusion, fission, or both necessitates the persistence of several independent chromosomal mutations, each one unlikely. By contrast all extant lemur karyotypes that have from 20 to 70 chromosomes are explained by only two karyotypic fissioning events (bottom).

Karyotypic fissioning offers the most parsimonious explanation for lemur chromosomal evolution and parsimony is attractive in terms of incorporating chromosomal mutations in a population. Such mutations are likely to be selected against (White, 1978) and in the event that they persist, are likely to be lost through hybrid extinction (Paterson, 1978). Within a single lemur family, the Lepilemuridae, diploid numbers range from 20 to 38. The conventional view of chromosomal evolution requires that at least nine sequential fusions become established, whereas karyotypic fissioning explains this range by means of a single event.

Karyotypic fissioning as a significant mechanism of chromosomal evolution that explains the apparent correlation between elevation in diploid numbers and adaptive radiation of mammals was first postulated by Todd (1967). Karyotypic fission theory postulates that all mediocentric chromosomes in a cell fission simultaneously during gametogenesis and that the resulting F1 hybrid potentially introduces a full complement of fission-generated acrocentric autosomes into the population (Todd, 1970, 1975, 1985, 1992). During

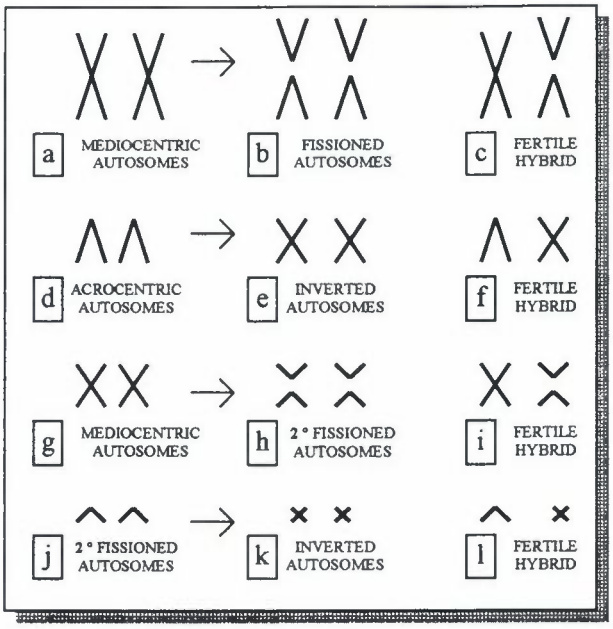

Figure 2. Homologous chromosomal combinations. During a karyotypic fissioning event, (a, g) each mediocentric chromosome yields (b, h) two smaller acrocentric chromosomes while retaining the same amount of DNA and the same genetic sequencing. During meiosis in the F1 hybrid, (c, i) fissioned acrocentric pairs synapse with homologous ancestral mediocentric chromosomes forming trivalents which result in corresponding acrocentrics segregating together. Chromosomal inversion requires two breaks to occur in a chromosome and the segment of DNA between the breaks to be reoriented in the opposite direction. Pericentric inversions (PIs) include the centromere in the inverted segment and subsequently contribute to karyotypic polymorphism by relocating the centromere and converting (d, j) acrocentric chromosomes to (e, k) mediocentric chromosomes or vice versa. (f, l) Karyological polymorphism resulting from PIs generally does not reduce fertility in mammal populations.

a karyotypic fissioning event (KFE), each mediocentric autosome yields two smaller acrocentric autosomes while retaining both gene sequence and total amount of DNA (Fig. 2a–c). A KFE alone would not result in gametic incompatibility, because fissioned acrocentric chromosomes are genetically compatible with nonfissioned homologues. For example, *Sus scrofa* has been found to be heteromorphic with a 1:2:1 ratio of diploid numbers 36, 37, and 38 depending on whether a pair of metacentric autosomes, a single metacentric autosome with two smaller acrocentric autosomes, or four smaller acrocentric autosomes are inherited (McFee, 1969). Neither viability nor fertility are reduced in matings and offspring of animals bearing any of the three karyotypes.

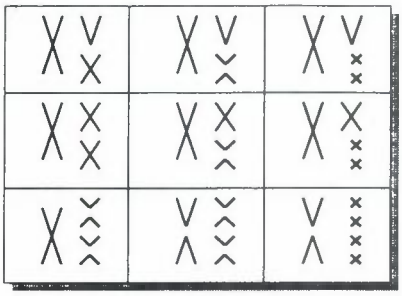

Figure 3. Because in mammals the quantity of chromatin is constant the amount here represented in each box is the same. Nonviable hybrids either infertile or with decreased fertility are generated by karyotypic fission followed by pericentric inversion. Difficulties apparently arise during synapsis of homologous DNA segments. Not all possibilities are shown.

Banding pattern studies indicate that homology exists between large metacentric chromosomes and smaller acrocentric pairs found in Eulemur karyotypes. DNA banding of chromosomes in *Eulemur fulvus* hybrids shows homologous pairing of 5 metacentric with 10 acrocentric autosomes in crosses between *E. f. rufus* (2N = 60) x *E. f. collaris* (2N=51) (Moses 1979). The sterility of intraspecific hybrids in crosses between *L. f. collaris* and *L. f. albocollaris* is addressed by Tattersall (1993) who reported variability in the degree of fertility in offspring resulting from *Eulemur fulvus* "subspecies" crosses; "we are witnessing here the results of a recent event of karyotypic innovation (or succession thereof) that has yet to result in full speciation." Fertile hybrids have resulted from interspecific crosses between *E. f. fulvus* and *E. mongoz* (2N=60) (Tattersall, 1993). Both fertile and sterile hybrids have resulted from interspecific crosses between *E. f. fulvus* (2N=60) and *E. macaco* (2N=44) (Tattersall, 1993; Dutrillaux and Rumpler, 1977; Albignac et al., 1971). Viable but sterile hybrids result from crosses between *E. fulvus fulvus* (2N=60) and *E. rubriventer* (2N=50) (Saint-Pie, 1970). Analysis of the hybrid chromosomal arrangement indicates that 5 submetacentrics in *E. rubriventer* pair with 10 acrocentric autosomes in *E. f. fulvus* (Rumpler and Dutrillaux, 1980). The fertility of interspecific hybrids indicates homology between the chromosomes in these distinct karyotypes in the Lemuridae.

3. Pericentric Inversions

Pericentric inversions (PIs) include the centromere in the inverted segment and subsequently contribute to karyotypic polymorphism by relocating the centromere and changing chromosome morphology without affecting the

family	range of diploid chromosome #'s	before fission	chromosome # in ancestral diploid karyotype		after fission
Lepilemuridae	**20 - 38**	20	20	→	**20 - 38**
Daubentoniidae	**30**	20 – 30	30	→	**30 - 58**
Lemuridae	**44 - 62**	32 – 38	32	→	**32 - 62**
Cheirogaleidae	**46, 66**	34 – 38	34	→	**34 - 66**
Indridae	**40 - 70**	36, 38	36	→	**36 - 70**
			38	→	**38 - 74**

Figure 4. Modern diploid ranges constrain postulated prefissioned ancestral chromosomal arrangements. The lowest diploid number in extant lemurs is 20. I hypothesize it closely approximates the ancestral condition. A KFE would yield diploid numbers in the range of 20 to 38. Secondary fissions explain diploid numbers higher than 38 found in three of the five lemur families. Fission events produce diploid numbers that range from the ancestral to double the number of autosomes plus the two ancestral sex chromosomes.

diploid number (Fig. 2d–f). PIs alone generally do not reduce fertility in mammalian populations. *Peromyscus maniculatus* has incorporated several pericentric inversions without genetically isolating populations that are heterozygous for inversions (Greenbaum and Reed, 1984). However, acquisition of PIs in fission-generated karyotypes may result in difficulty during synapsis in pairing the inverted chromosomes to their homologues; the result may be gametic incompatibility leading to reproductive isolation when both rearrangements are prevalent in a population (Fig. 3).

4. Ancestral Chromosomal Arrangements

To determine ancestral chromosomal arrangements using karyotypic fission theory one first looks at the familial diploid range and asks what ancestral morphotype could have yielded the modern diversity through karyotypic fissioning (Fig. 4). Lemur diploid numbers range from 20–70, yet the ranges found within families are more restricted: e.g. Lepilemuridae 2N=20–38, Daubentoniidae 2N=30, Lemuridae 2N=44–62, Cheirogaleidae 2N=46 and 66 and Indridae 2N=40–70. A fissioning event in the proposed pre-fissioned ancestral lemur condition could yield diploid numbers ranging from 20 to 38. Secondary fissioning events are postulated to explain diploid numbers higher than 38 found in three of the five lemur families. Lemurid diploid numbers can be generated by a secondary fissioning event in an ancestral chromosomal complement having a 2N no lower than 32. Cheirogaleid diploid numbers can

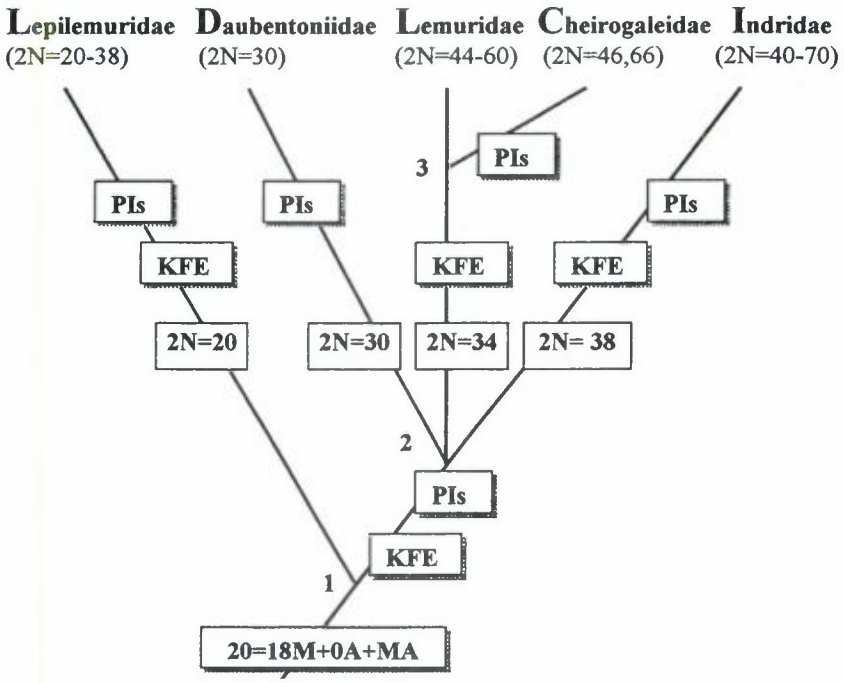

Figure 5. Karyological phylogeny of lemurs. Lemur karyotypes can be most parsimoniously explained as a product of only a few karyotypic fissioning events (KFEs) followed by pericentric inversions (PIs). This figure shows the hypothetical scenario for karyological evolution in lemurs. Node 1. Lemuriformes are derived from an ancestral diploid number of 20. The prefissioned lemuriform ancestral karyotype (2N=20) with all mediocentric autosomes is maintained in the Lepilemuridae and is closely approximated in *Lepilemur ruficaudatus*. A KFE with varying retention of mediocentric linkages and incorporation of pericentric inversions generates all modern lepilemurid karyotypes. Node 2. A KFE in the karyotype (2N=20) could yield polymorphic diploid numbers, three of which (2N=30, 34 and 38) generate the karyotypes of all extant species in the Daubentoniidae, Lemuridae, Indridae, and Cheirogaleidae. The karyotype of *Daubentonia madagascarensis* (2N=30) has 24 metacentric and 4 acrocentric autosomes and can be explained by incorporation of 8 autosomal pericentric inversions following the primary KFE. Indrid karyotypes can be explained by a KFE in a chromosomal arrangement (2N=38) having all mediocentric autosomes. This hypothetical arrangement can be generated from the earlier KFE in the ancestral arrangement 2N=20 with subsequent acquisition of pericentrically inverted autosomes. Node 3. A KFE in a population with karyological polymorphism (2N=34) having 30 mediocentric and 2 acrocentric autosomes or 32 mediocentric and 0 acrocentric autosomes generates the karyotypes of Lemuridae and Cheirogaleidae. All lemurid karyotypes can be derived from the karyotype (2N=34) having 2 acrocentric autosomes. This hypothetical karyotype can be explained by the earlier KFE in a karyotype (2N=20) generating the karyotype (2N=34) with 4 mediocentric and 28 acrocentric autosomes and incorporation of 13 autosomal PIs.

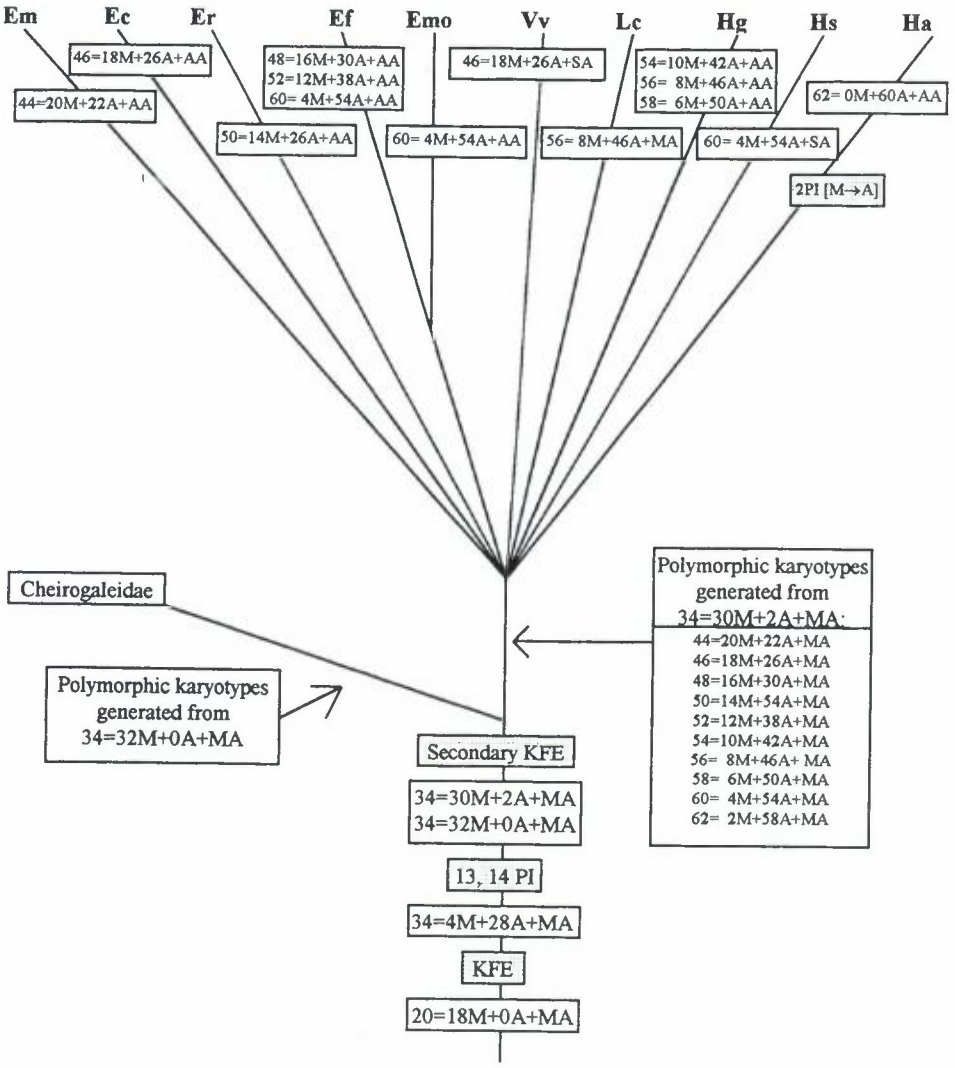

Figure 6. Lemurid karyological evolution. A secondary karyotypic fissioning event explains all modern chromosomal arrangements. Key: Em = *Eulemur macaco*, Ec = *Eulemur coronatus*, Er = *Eulemur rubriventer*, Emo = *Eulemur mongoz*, Ef = *Eulemur fulvus*, Ha = *Hapalemur aureus*, Hs = *Hapalemur simus*, Hg = *Hapalemur griseus*, Vv = *Varecia variegata*, Lc = *Lemur catta*. Karyotype morphology is represented as 2N = M (mediocentric autosomes) + A (acrocentric autosomes) + MA, SA, or AA (the final pair refers to the sex chromosomes), S = submetacentric, KFE = karyotypic fissioning event, PI = pericentric inversion, [A→M] = acrocentric to mediocentric, [M→A] = mediocentric to acrocentric. Major events lightly shaded.

be generated from fissioning a 2N of 34, 36 or 38. Indrid diploid numbers can be explained by fissioning a diploid number of 36 or 38. Chromosome morphology, DNA banding, and PIs provide further constraints on the ancestral condition that best explains each family's karyotypes.

5. Lemur Phylogeny

This karyological phylogeny suggests that all extant lemur karyotypes within the five families can be parsimoniously generated by four fissioning events coupled with intercalated pericentric inversions (Fig. 5). The first fissioning event generated the karyotypic diversity that later became fixed in the ancestral stocks of four lemur families. The ancestral stock of the Lepilemuridae was not affected by this event, but did experience a later independent fissioning event.

The Lepilemuridae includes seven species (*Lepilemur dorsalis, L. edwardsi, L. leucopus, L. microdon, L. mustelinus, L. ruficaudatus,* and *L. septentrionalis*) for which all karyotypes are readily explained by a karyotypic fissioning event in a pre-fissioned mediocentric arrangement having a diploid number of 20. The family Daubentoniidae has only one extant species, *Daubentonia madagascariensis,* the aye-aye, and its karyotype can be explained as resulting from fissioning the postulated ancestral arrangement. The family Lemuridae includes ten species of diurnal and cathemeral (active both day and night) lemurs: *Lemur catta, Eulemur coronatus, E. macaco, E. mongoz, E. rubriventer, E. fulvus, Varecia variegata, Hapalemur griseus, H. aureus,* and *H. simus* (Tattersall, 1988; Mittermeier et al., 1994). All lemurid karyotypes are considered to be derived from a pre-fissioned arrangement having a diploid number of 34 with two acrocentric autosomes. There are eight species in Cheirogaleidae and all cheirogaleid karyotypes are considered to be derived from a pre-fissioned complement having a diploid number of 34 with all mediocentric autosomes. A single secondary KFE explains all karyotypes in both the Lemuridae and the Cheirogaleidae. The family Indridae has six species (*Avahi laniger, A. occidentalis, Indri indri, Propithecus verreauxi, P. diadema,* and *P. tattersalli*) and a separate secondary fissioning in a mediocentric arrangement having a diploid number of 38 explains all indrid karyotypes. Without recourse to other chromosomal rearrangements, fissioning alone accounts for both the diploid numbers and the karyological morphology exhibited by many species of lemurs (particularly in the family Lemuridae).

6. Lemuridae Phylogeny

A closer look at the karyotypic evolution of one of the five lemur families

clearly illustrates the elegance and simplicity of the karyotypic fission theory. Lemurid diploid numbers range from 44 to 62. A secondary fissioning event is postulated to have occurred in an ancestral arrangement having a diploid number of 30 with 2 acrocentric autosomes (Fig. 6). That lemurids retain one pair of acrocentric autosomes generated from the primary fissioning event is consistent with actual karyotype morphology. DNA banding studies indicate that homology exists between large metacentric chromosomes and smaller acrocentric pairs found in *Eulemur* karyotypes (Dutrillaux and Rumpler, 1977; Rumpler and Dutrillaux, 1976, 1978, 1979, 1980; Rumpler et al., 1983). KFEs usually do not alter a population's sex chromosomes yet lack of uniformity makes it necessary to address the X-chromosomes in lemurid karyotypes. *Varecia variegata* and *Hapalemur simus* have submetacentric X-chromosomes; *Lemur catta* has a metacentric X chromosome, and all other lemurid species have acrocentric X-chromosomes. Acrocentric sex chromosomes are not fissioned products. DNA banding patterns indicate that the difference seen between these sex chromosomes resulted from pericentric inversions (Rumpler and Dutrillaux, 1978, 1979).

The transition from the postulated ancestral lemuriform arrangement (2N=20) to the modern karyotype of *Eulemur macaco* is diagrammed (Fig. 7). A primary KFE in the postulated lemuriform ancestral condition (a) could yield an arrangement (2N=34) having 4 mediocentric and 28 acrocentric autosomes (b). PI of 13 acrocentric autosomes [A→M] results in an arrangement (2N=34) having 30 mediocentric and two acrocentric autosomes (c). An additional autosomal PI [A→M] yields an arrangement with all mediocentric autosomes, from which all cheirogaleid karyotypes can be derived. The karyotype of *E. macaco* is explained as resulting from the retention of two large pair of ancestral mediocentric linkages, one pair of acrocentric autosomes resulting from the primary fissioning event, eight pair of smaller mediocentric autosomes resulting from subsequent PI, and ten pairs of small acrocentric autosomes resulting from the postulated secondary fissioning event.

7. A Mechanism for Karyotypic Fissioning

Recent research on the behavior, structure and physiology of chromosomes and their kinetochores supplies a mechanism for karyotypic fissioning. During mitotic cell reproduction chromatids segregate by attaching to the spindle. Kinetochores, microtubule-organizing centers on chromosomes, are necessary for all normal mitoses; they are essential in spindle formation. Chromosomes lacking kinetochores fail to attach to the mitotic spindle, and aneuploidy results. Kinetochores are disc-shaped proteinaceous structures where microtubules bind. They are visible by electron microscopy on the surface of the

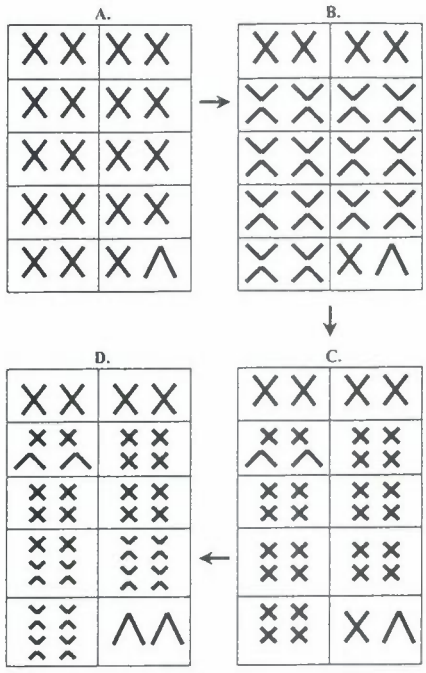

Figure 7. Chromosomal evolution of *Eulemur macaco* (2N=44). A) The ancestral chromosomal arrangement for all lemurs is postulated as having all mediocentric autosomes (2N=20). B) A fissioning event in this postulated ancestral arrangement could generate a karyotype (2N=34) by retaining four ancestral linkages and fissioned products in the remaining autosomes. C) The incorporation of 13 pericentric inversions yields an arrangement (2N=34) from which all lemurid karyotypes can be derived. D) The karyotype of *E. macaco* is explained as resulting from the retention of 2 large pair of ancestral mediocentric linkages, one pair of acrocentric autosomes resulting from the primary fissioning event, 8 pairs of smaller mediocentric autosomes resulting from subsequent pericentric inversions, and finally 10 pairs of small acrocentric autosomes resulting from the postulated secondary fissioning event.

centromere (Earnshaw and Tomkiel, 1992). Cleveland (1957) showed that kinetochores reproduce and segregate by spindle attachment even when detached from chromosomes. Cleveland (1963) also showed that, without kinetochores, chromosomes reproduce themselves but do not separate. The kinetochore functions independently from the chromosome with which it is associated, and kinetochore division can be independent of chromosome reproduction. Kinetochore reproduction is synchronized during the S stage of interphase when sister chromatids replicate (Earnshaw and Tomkiel, 1992). The fact that centromeres normally undergo simultaneous reproduction but that

they may reproduce independently from the rest of the chromatin indicates a high plausibility that (with any signal delaying cytokinesis) all chromosomes in a karyotype can undergo an extra round of centromeric reproduction to yield a complete set of fissioned chromosomes with functional kinetochores.

The regulatory mechanism ensuring proper segregation of acrocentric pairs may be found in kinetochore protein research. In mammalian and insect cells certain kinetochore proteins are phosphorylated before the chromosomes attach to spindle. They dephosphorylate after proper chromosomal attachment (Nicklas, 1997). During metaphase the kinetochore proteins of chromosomes that are misattached remain phosphorylated. Anaphase does not ensue until kinetochore protein dephosphorylation occurs on all the chromosomes. Tension caused by spindle attachment directly effects protein phosphorylation. Micromanipulation experiments conducted by Nicklas show that tension-sensitive-phosphorylation of kinetochore proteins signal the onset of anaphase. Fission-generated acrocentric chromosomes, as seen in cells of hybrid animals, synapse with homologous mediocentric chromosomes to form trivalents. Regulation of segregation of fission-generated acrocentric pairs at least in part is by kinetochore protein dephosphorylation. Kinetochores of acrocentric pairs still sensitive to tension, will dephosphorylate and will tend to segregate normally from their mediocentric homologues. Proper segregation of acrocentric pairs will occur when the amount of tension applied to kinetochores of acrocentric pairs facing the same pole is the same as that of the mediocentric homologue attached to the opposite pole. If a single acrocentric chromosome is pulled in the same direction as its mediocentric homologue the tension will be detected as incorrect and dephosphorylation will not occur.

Previous theories of fusion, all ad hoc, require far more independent events than fission theory to derive all 29 lemur karyotypes here. The ancestor to the genetic determinant of the centriole-kinetosome and centromeres is a motile eubacterium, by hypothesis a spirochete according to the serial endosymbiosis theory (Margulis, 1993). Endocellular symbionts tend to reproduce out of synchrony from their hosts even in co-evolved eubacterial symbiotic associations like those of mitochondria and plastids. Karyotypic fission theory where centromere "splitting" is understood as rapid centromeric residual reproduction of a once-foreign (once spirochete eubacterial) genome is entirely consistent with a "symbiogenetic" rather than a "direct filiation" concept of eukaryotic cell evolution.

8. Conclusion

Todd's karyotypic fission theory is an underappreciated and elegant theory of mammalian chromosomal evolution. Chromosomal polymorphism is common

in mammalian taxa. However, many researchers assume that in karyological evolution 1) ancestral species had higher diploid numbers than more derived species, and 2) that small acrocentric chromosomes fuse to form larger mediocentric chromosomes. By contrast karyotypic fission theory, far more successful in explanation of extant lemur chromosome patterns, postulates 1) a low ancestral diploid number for all mammals, and 2) that diversity in mammalian karyotypes is generated by small numbers of fissioning events that occurred because of differential rates of reproduction of kinetochores (and their associated centromeric DNA) that evolved from former bacterial symbionts.

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