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Mouse Lemur Phylogeography Revises a Model of Ecogeographic Constraint in Madagascar

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Introduction

Phylogeographic methods involve determining the historical relationships among gene lineages with attention to the contemporary spatial distribution of those lineages. In other words, the methodology entails deriving a gene tree for an array of individuals and/or taxa and then mapping that tree onto the geographic localities whence the individual DNA samples were collected. By so doing, one can potentially examine the effects of putative geographic barriers to gene flow. If geographic samples are reciprocally monophyletic with respect to a putative geographic barrier (Fig. 1a), then that barrier is often inferred to have inhibited the dispersal of individuals and their genes. If, on the other hand, the geographic samples are not reciprocally monophyletic (Fig. 1b), then we can infer either that the barrier is not a barrier at all, or that the barrier arose too recently yet to be recorded in the genetic data. Because these methods have a concern both for spatial patterning of individual alleles, and for their historical relationship to other homologous alleles, phylogeography has emerged as the theoretical bridge that unites the traditionally distinct fields of phylogenetics and population genetics (Avise et al., 1987; Avise, 1989).

To date, phylogeographic studies have focused primarily on mitochondrial DNA (mtDNA) (e.g., Avise et al., 1992; Da Silva, Patton, 1993; Taberlet, Bouvet, 1994; Ward, 1997; Avise, Walker, 1998; Eizirik et al., 1998; Lucchini, Randi, 1998; Bensch, Hasselquist, 1999). Advantages of mtDNA for phylogeographic studies were highlighted by Avise et al. (1987) in their seminal paper: mtDNA is distinctive yet ubiquitously distributed, is easy to isolate, amplify and sequence, has a simple genetic structure, is non-recombining and uniparentally inherited, and evolves rapidly enough to provide information at even intra-populational levels. Yet, there are regions conserved enough to be informative at much higher taxonomic levels. Despite all of these advantages, reliance on mtDNA alone has been criticized (Pamilo and Nei, 1988; Hare, 2001; Ballard and Whitlock, 2004). Because the mtDNA genome is non-recombining, one is examining a single locus, no matter how many mitochondrial genes one chooses to sequence. And, because it is maternally inherited, it is possible that organismal mating patterns (e.g., strong female philopatry) can potentially skew the results (Hoelzer, 1997). Several empirical and theoretical studies have indicated, however, that this may not be as problematic as has been suggested (Avise, 1992;

Moore, 1995). Also, the problems associated with nuclear DNA markers are not trivial. Avise (1998; 2000) summarizes these as two: 1) it is difficult to isolate single alleles from a diploid organism and 2) it is difficult to find markers that are accumulating mutations rapidly enough for fine-scale resolution, but are free of recombination. Consequently, even though nDNA markers are increasingly more common in phylogeographic studies (Hare, 2001), their use is still limited and largely in conjunction with mtDNA markers.

Given the various issues described above, one is typically faced with a single gene tree (usually mtDNA) for a taxonomically-focused group of organisms. Even though striking patterns might present themselves in such an analysis, one might well ask, "how much about geographic structuring can safely be inferred from such limited data?" Clearly, inferences must be limited, especially given that stochastic processes such as isolation by distance can readily create similar patterns (Irwin, 2002). As a means for extending the power of such analyses, the newly-emerging field of comparative phylogeography has been offered as a method for the investigation of "landscape evolution", including patterns of gene flow and genealogical vicariance, even in the absence of an a priori hypothesis of localized barriers to gene flow (Bermingham, Moritz, 1998). DaSilva and Patton (1998) detail the logic of the approach, which essentially involves the comparison of phylogeographic patterns for multiple co-distributed species. First, the observation of reciprocally-monophyletic groups offers support for long-term spatial barriers to gene flow. Second, geographically concordant phylogenetic gaps for different taxa can identify common boundaries and/or historical events. Thus, these multiple vicariant biogeographic histories can be used as evidence for interpreting the biogeographic history of a region. Thus far, studies employing this method are growing in number, and show mixed results. While some have shown congruent patterns among the organisms, thereby indicating strong biogeographic patterns (Da Silva, Patton, 1998; Moritz, Faith, 1998; Waltari et al., 2004), others have not (Zink, 1996; Taberlet et al., 1998; Hofreiter et al., 2004).

Landscape Evolution in Madagascar

Madagascar has long been recognized as an island of rare floral and faunal diversity. At present, it lies approximately 350 kilometers to the east of Africa at the narrowest point of the Mozambique Channel and is otherwise completely isolated from

other significant landmasses. The complex relationship between geological history and geographic isolation has conspired to create its unique assemblage of organisms. The Malagasy flora and fauna are a fascinating mix of singularity and diversity, singular due to the island's ancient isolation, and diverse due to the complexity of its topography and ecology. Due to its large surface area, and its varied assortment of microclimates and habitats, it is often referred to as a mini-continent (de Wit, 2003). Much of Madagascar's ecological variation relates to its sharply asymmetrical topography. The eastern edge, where it was once conjoined with India, is ruggedly mountainous, abruptly rising from the Indian Ocean to attain elevations of 2000 m, and is characterized by moist evergreen rainforest. Altitudes gradually diminish to sea level in the west, where the vegetation is predominated by dry deciduous forest. There, rainfall is sharply lower, with the extreme southwest receiving less than 35 cm/yr of rainfall. The intervening central plateau is comprised primarily of depauperate grassland. Preliminary analyses indicate that the inherent dissimilarities in topography between eastern and western Madagascar have important bearing on the biogeography of these two portions of the island (Goodman, Ganzhorn, 2004a; Goodman, Ganzhorn, 2004b).

Early in the 20th century, the prevailing view of Madagascar's pre-settlement landscape was that human habitation was entirely responsible for the abrupt disjunction between east and west, and that prior to the arrival of humans, Madagascar was entirely covered by closed forest formations with wildfire virtually absent (Humbert, 1927; Perrier de la Bâthie, 1927). Analyses of palynological and geological data show, however, that much of Madagascar's central plateau has long been characterized by a mosaic of woodlands, shrublands, and grasslands (Burney, 1997). Moreover, analysis of Madagascar's topography, in concert with climatological and paleogeographic data, indicate that the "eastern edge" watershed has concentrated rain on the east coast and desiccated the west since the late Cretaceous (Wells, pers. com.). Superimposed on this east/west asymmetry is a north/south climatic gradient, most obvious in the west, wherein the island tends to become progressively drier in a north to south progression. In sum, these data suggest that disparate eastern and western ecological communities might have provided a separate suite of ecogeographic

characteristics in which terrestrial vertebrates were able to diversify into the variety of forms that we observe today.

Inferring Big Patterns with Small Primates

The model of ecogeographic constraint summarized above presents a number of questions that can potentially be addressed with phylogeographic data. Most obviously, the long-term separation and ecological distinction between eastern and western habitats would suggest that organisms with limited means of dispersal (e.g., terrestrial vertebrates) that are distributed along both coasts might show strong historical roots within their respective geographic locales. Intuitively, congeneric species distributed along one coast would be more closely related to each other than to congeners distributed along the opposite coast and the range of individual species within a genus would not extend to both coasts. This pattern of east/ west distribution is found commonly in range distributions for the majority of lemur species (Mittermeier et al., 1994). In fact, there are few examples of lemurs that do not exhibit a disjunct distribution (see Mittermeier et al., 1994; Simons, 1993; Sterling, 1994). It is important to note, however, that with the inclusion of subfossil specimens, many exceptions to the general pattern of east/ west species disjunction can be observed, also with evidence for a potential dispersal corridor between east and west across the central highlands (Godfrey et al., 1999).

Until the late 1970's, Microcebus was considered monotypic by most authorities, containing only the species murinus (Schwarz, 1931). Upon broader geographic sampling and increased research activity, researchers who were studying mouse lemur populations reached the conclusion that there were actually two species in the genus (Martin, 1972; Martin, 1973; Petter et al., 1977). One was a dry-adapted form that was restricted to the western portions of Madagascar, and the other was a wet-adapted form found in the eastern forests. The first, which retained the name murinus, was typified as a long-eared gray form, and the second, that was given the name M. rufus, was typified as a short-eared reddish form. Thus, the two species taxonomy emphasized both ecogeographic and morphological distinctions between the two mouse lemur types. Martin (1973) made particular note of the differing habitats and ecological constraints that appeared to define the two species, with M. murinus inhabiting dry

deciduous and spiny desert forest and specializing on insectivory, and M. rufus inhabiting humid rain forest and showing dietary tendencies towards omnivory. Thus, the idea that both ecological and biogeographic mechanisms maintain species separation was an implicit assumption of the two-species taxonomy.

The two-species classification remained stable until recently. Within the past few years, however, that classification has been radically altered. The revision began with the discovery that two distinct mouse lemur forms occur in non-interbreeding sympatry at the western locality of the Kirindy Forest (Kirindy/CFPF). One is the typical M. murinus of dry forests and the other is a distinctly smaller rufus-colored animal. The authors of that study (Schmid, Kappeler, 1994) concluded that the second form fit with the original diagnosis of M. myoxinus. Subsequently, a much larger mouse lemur type, also sympatric with M. murinus, was described from the northwest and designated as M. ravelobensis. More recently still, the number of mouse lemur species has been doubled by a morphological study that sampled broadly throughout western localities (Rasoloarison et al., 2000).

In collaboration with the authors of that most recent study, Yoder and several colleagues undertook a mtDNA phylogeographic study of the same western populations along with several eastern populations (Yoder et al., 2000). The purpose of the study was both to test the species designations proposed by Rasoloarison et al. (2000) and to identify the historical relationships among the various populations sampled by that study. To accomplish these goals, we initially adopted a null hypothesis of species homogeneity and accordingly selected a rapidly-evolving mtDNA marker likely to show variation at the intraspecific level. We sequenced an approximately 500 base pair segment of the mtDNA control region, homologous with the hypervariable region 1 region in humans (HV1), for all 118 individuals sampled by our study. Samples of M. rufus from two eastern localities were included and originally intended to serve as outgroups to the western populations. HV1 showed surprisingly high levels of sequence variation and yielded a tree wherein there were well-resolved clades that are perfectly congruent with the morphological species designations of Rasoloarison et al. (2000) but whose interrelationships could not be determined due to poor internal resolution (Fig. 2). The latter result is presumably due to saturation, and

in fact, was our first indication that the null hypothesis of a single species might be incorrect. The HV1 tree was also surprising in that the two populations of M. rufus do not form a clade, and instead, are both nested within clades that contain western populations. To attempt better resolution of deeper nodes, we sub-sampled individuals from each of the well-supported HV1 clades and sequenced them for the more conserved cytochrome oxidase subunit II (684 bp) and cytochrome *b* (1140 bp) genes.

The combined analysis of the three mtDNA markers yields a tree in which the nine terminal clades from the HV1-only analysis are identically resolved (Fig. 3) and has the additional strength of resolving two deep clades with strong support, thereby allowing a test of the east/west biogeographic constraint hypothesis. If this hypothesis held, we would expect a phylogeographic scenario much like the one illustrated in Figure 4 wherein individuals sampled from eastern localities would form one clade, and those from western localities would form another. An entirely different pattern emerged, however. As had been suggested by the HV1 analysis, there is no clear grouping into eastern and western clades. Rather, the populations sampled appear to form northern and southern clades (Fig. 5). Aside from the departure from expectation, this result is surprising in that it is difficult to surmise what is, or could have been, the biogeographic barrier separating northern and southern mouse lemur communities. One possibility is highlighted by Pastorini et al., (2003) who demonstrated the importance of rivers as barriers to gene flow for multiple populations of lemurs along the west coast. In that study, the authors found that both the Tsiribihina and Betsiboka Rivers to be significant isolating mechanism for a number of lemur species and subspecies along the west coast of Madagascar.

Testing the Reality of the Pattern

To briefly summarize, mouse lemurs show historical relationships that indicate close connections between eastern and western populations across similar latitudes, thereby falsifying any notion that the ecogeographic disjunction between eastern and western habitats serves as an insurmountable barrier to dispersal. Moreover, an unexpected pattern of northern and southern clades emerged. Our results are mirrored to some extent by a study by Pastorini et al., (2001b) in which she also found that putative M. rufus are in a clade with M. ravelobensis from the northwest that excludes

M. murinus, also from the west. That study did not address the north/south pattern observed in our study, however, due to its limited geographic sampling. On the other hand, the pattern of a north/south disjunction observed in the Yoder et al. (2000) study may simply be a consequence of limited sampling of M. murinus from north of the Tsiribina River. To sufficiently test these biogeographic patterns in Microcebus, we need to expand our sampling of mouse lemurs from the northwest and from all regions of the east (work in progress) and include subfossil specimens from the central plateau (Godfrey et al., 1997). Furthermore, we need to expand our genetic sampling beyond the confines of the mitochondrial locus. To that end, we are assembling sequence data from a suite of independently-segregating nuclear loci. Thus far, analysis of these data further indicate the lack of any east/west structuring of mouse lemur populations or species (Heckman et al., in prep.).

The indication of a primary north/south biogeographic division in Madagascar is suggested by recent studies of other Malagasy lemurs. Within the species Eulemur fulvus, a north/south split is seen between subspecies marked in the west by the Betsiboka River (Pastorini et al., 2000), rather than the Tsiribina River as in the Yoder et al. (2000) study. In addition, in a study of the Indridae (Pastorini et al., 2001b) show an essential split of P. verreauxi into northern and southern clades also separated by the Betsiboka River. Looking more broadly, an example of biogeographic pattern outside of lemurs is found in the chameleon genus Calumma, where strong evidence for regional structuring of southern populations from western and northern populations is found. In the Calumma study, however, sampling locations are limited and results may represent a pattern of isolation by distance (Russell et al., in prep.). This work is ongoing, and as with the mouse lemur study, additional intervening populations are being sampled.

In the meantime, the mouse lemur data confirm the fact that the biogeographic and ecological separation between western and eastern habitats has not been particularly restrictive to interpopulation gene flow among mouse lemur populations. Future studies will focus on increased sampling of taxa, localities, and ecosystems throughout Madagascar. Such multi-layered analysis is the key to revealing patterns that are of universal impact, versus those that are merely idiosyncratic.

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Bibliography

- Avice JC (1989) Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**, 1192-1208.
- Avice JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63**, 62-76.
- Avice JC (1998) The history and purview of phylogeography: a personal reflection. *Molecular Ecology* **7**, 371-379.
- Avice, J.C. 2000. Phylogeography: The history and formation of species. Harvard University Press. Cambridge, Massachusetts.
- Avice JC, Alisauskas RT, Nelson WS, Ankney CD (1992) Matriarchal population genetic structure in an avian species with female natal philopatry. *Evolution* **46**, 1084-1096.
- Avice JC, Arnold J, Ball RM, *et al.* (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**, 489-522.
- Avice JC, Walker D (1998) Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London, Series B* **265**, 457-463.
- Ballard, J.W.O., and M.C. Whitlock. 2004. The incomplete natural history of mitochondria. *Molecular Ecology*, 13:729-744.
- Bensch S, Hasselquist D (1999) Phylogeographic population structure of great reed warblers: an analysis of mtDNA control region sequences. *Biological Journal of the Linnean Society* **66**, 171-185.
- Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology* **7**, 367-369.
- Burney DA (1997) Theories and facts regarding Holocene environmental change before and after human colonization. In: *Natural Change and Human Impact in Madagascar* (eds. Goodman SM, Patterson BD), pp. 75-89. Smithsonian Institution Press, Washington, D.C.
- Da Silva MNF, Patton JL (1993) Amazonian phylogeography: mtDNA sequence variation in arboreal echimyid rodents (Caviomorpha). *Molecular Phylogenetics and Evolution* **2**, 243-255.

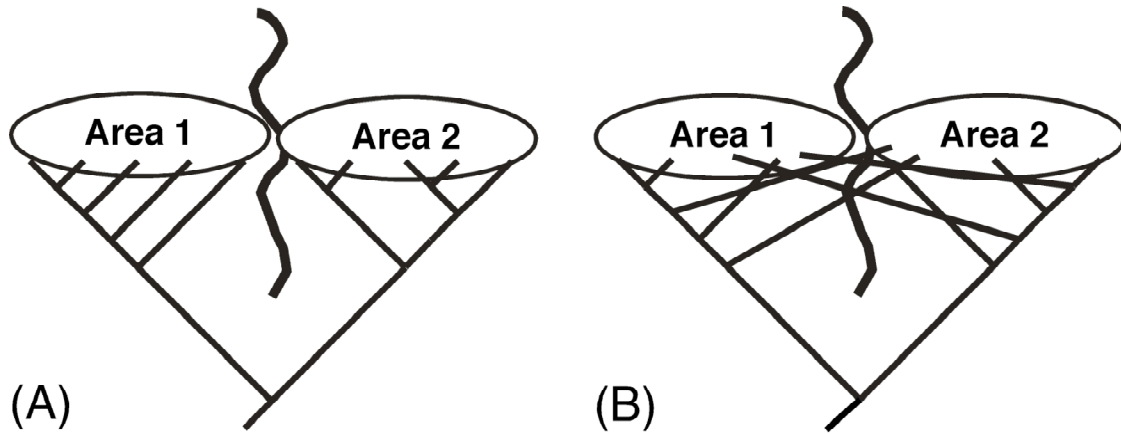
- Da Silva MNF, Patton JL (1998) Molecular phylogeography and the evolution and conservation of Amazonian mammals. *Molecular Ecology* **7**, 475-486.
- de Wit M (2003) Madagascar: heads it's a continent, tails it's an island. *Annual Review of Earth and Planetary Sciences* **31**, 213-248.
- Eizirik E, Bonatto SL, Johnson WE, *et al.* (1998) Phylogeographic patterns and evolution of the mitochondrial DNA control region in two neotropical cats (Mammalia, Felidae). *Journal of Molecular Evolution* **47**, 613-624.
- Godfrey, L.R., W.L. Jungers, E.L. Simons, *et al.* 1999. Past and present distributions of lemurs in Madagascar. In Rakotosamimanana *et al.*, (eds.) New directions in lemur studies. Kluwer Academic/ Plenum Publishers, New York.
- Goodman SM, Ganzhorn J (2004a) Biogeography of lemurs in the humid forests of Madagascar: The role of elevational distribution and rivers. *Journal of Biogeography* **31**, 47-55.
- Goodman SM, Ganzhorn J (2004b) Elevational ranges of lemurs in the humid forests of Madagascar. *International Journal of Primatology* **25**, 331-350.
- Hammer MF, Karafet T, Rasanayagam A, *et al.* (1998) Out of Africa and back again: a nested cladistic analysis of human Y chromosome variation. *Molecular Biology and Evolution* **15**, 427-441.
- Hare, M.P. 2001. Prospects for nuclear gene phylogeography. *Trends in Ecology and Evolution*, 16:700-706.
- Hoelzer, G.A. 1997. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees revisited. *Evolution*, 51:62-626.
- Hofreiter, M., D. Serre, N. Rohland, *et al.* 2004 Lack of phylogeography in European mammals before the last glaciation. *Proceeding of the National Academy of Sciences of the USA*, 101:12963-12968.
- Humbert H (1927) Destruction d'une flore insulaire par le feu: principaux aspects de la végétation à Madagascar. *Mémoires de l'Académie Malgache* **5**, 1-80.
- Irwin DE (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution Int J Org Evolution* **56**, 2383-2394.
- Lucchini V, Randi E (1998) Mitochondrial DNA sequence variation and phylogeographical structure of rock partridge (*Alectoris graeca*) populations. *Heredity* **81**, 528-536.

- Martin RD (1972) A preliminary field-study of the lesser mouse lemur (*Microcebus murinus* J.F. Miller, 1777). *Zeitschrift für Tierpsychologie* **Suppl. 9**, 43-89.
- Martin RD (1973) A review of the behaviour and ecology of the lesser mouse lemur (*Microcebus murinus* J.F. Miller, 1777). In: *Comparative Ecology and Behaviour of Primates* (eds. Michael RP, Crook JH), pp. 1-68. Academic Press, London.
- Mittermeier, R.S., I. Tattersall, W.R. Knonstant et al. 1994 Lemurs of Madagascar. Conservation International, Washington D.C.
- Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49**, 718-726.
- Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology* **7**, 419-429.
- Pamilo P, Nei M (1988) Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5**, 568-583.
- Pastorini, J., M.R.J. Forstner, R.D. Martin. 2000 Relationships among brown lemurs (*Eulemur fulvus*) based on mitochondrial DNA sequences. *Molecular phylogenetics and evolution*, 16:418-429.
- Pastorini J, Forstner MRJ, Martin RD (2001) Phylogenetic history of sifakas (*Propithecus*: Lemuriformes) derived from mtDNA sequences. *American Journal of Primatology* **53**, 1-17.
- Pastorini, J., M.R.J. Forstner, and R.D. Martin. 2001a. Phylogenetic history of sifakas (*Propithecus*: Lemuriformes) derived from mtDNA sequences. *American Journal of Primatology*, **53**:1-17.
- Pastorini, J., R.D. Martin, P. Ehresmann, et al. 2001b. Molecular phylogeny of the lemur family Cheirogaleidae (Primates) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 19:45-56.
- Pastorini, J., U. Thalmann, R.D. Martin. 2003. A molecular approach to comparative phylogeography of extant Malagasy lemurs. *Proceedings of the National Academy of Science*, 100:5879-5884.
- Perrier de la Bâthie H (1927) La végétation malgache. *Annals du Muséum Colonial, Marseille* **9**, 1-266.
- Petter J-J, Albignac R, Rumpler Y (1977) Mammifères Lémuriens (Primates Prosimiens). In: *Faune de Madagascar*, pp. 1-513. ORSTOM and CNRS, Paris.

- Rasoloarison RM, Goodman SM, Ganzhorn JU (2000) Taxonomic revision of mouse lemurs (Microcebus) in the western portions of Madagascar. *International Journal of Primatology* **21**, 963-1019.
- Schmid J, Kappeler PM (1994) Sympatric mouse lemurs (Microcebus spp.) in western Madagascar. *Folia Primatologica* **63**, 162-170.
- Schwarz E (1931) A revision of the genera and species of Madagascar Lemuridae. *Proceedings of the Zoological Society of London* **1931**, 399-428.
- Simons, E.L. 1993. Discovery of the western aye-aye. *Lemur News*, 1:6.
- Sterling, E. 1994. Taxonomy and distribution of *Daubentonia*: A historical perspective. *Folia Primatologica*, 62:8-13.
- Taberlet P, Bouvet J (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear Ursus arctus in Europe. *Proceedings of the Royal Academy of London, B* **255**, 195-200.
- Taberlet P, Fumagalli L, Wust-Saucy AG (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* **7**, 453-464.
- Ward RH (1997) Phylogeography of human mtDNA: an Amerindian perspective. In: *Progress in Population Genetics and Human Evolution* (eds. Donnelly P, Tavaré S), pp. 33-53. Springer-Verlag, New York.
- Yoder AD, Rasoloarison RM, Goodman SM, *et al.* (2000) Remarkable species diversity in Malagasy mouse lemurs (primates, Microcebus). *Proc Natl Acad Sci U S A* **97**, 11325-11330.
- Zink RM (1996) Comparative phylogeography in North American birds. *Evolution* **50**, 308-317.

Figure 1:

Schematic diagrams comparing models in which haplotype relationships show a) reciprocal monophyly with respect to a putative biogeographic barrier (wavy line) between two localities versus b) those that do not show reciprocal monophyly, indicating gene flow across barrier.



Yoder (Fig. 1) ↑

Figure 2:

Distance tree of 118 mouse lemur mtDNA haplotypes derived from 580 bp alignment of control region sequence homologous with HV1 in humans. Individuals are identified by unique laboratory extraction number (Yoder Lab Extraction; YLE) and by locality. Tree was generated in PAUP* 4.0b4a (PPC) (Swofford, 1998) by using HKY85 correction and weighted least squares (power = 2) algorithm. Branches are proportional to expected number of changes per site. Numbers on branches indicate statistical support from 100 bootstrap replicates of the "fast" stepwise-addition algorithm for species-level clades. Tree was rooted with *Propithecus* and *Varecia* (not shown).

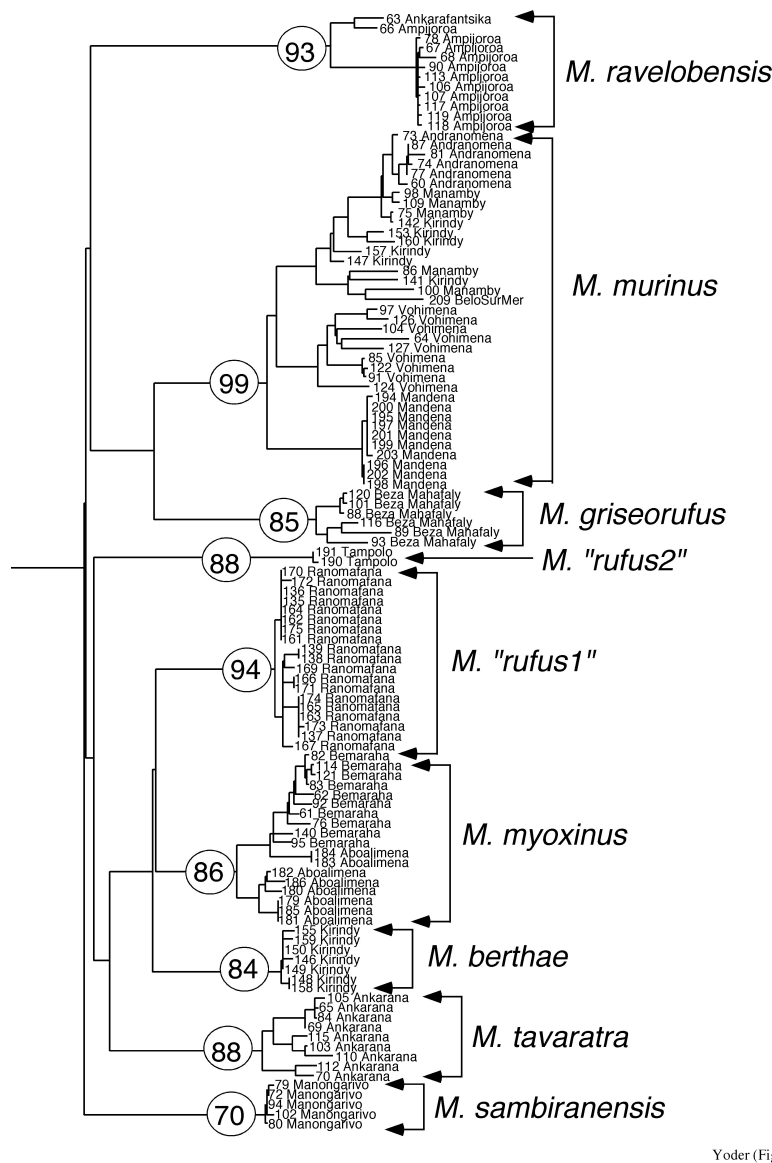
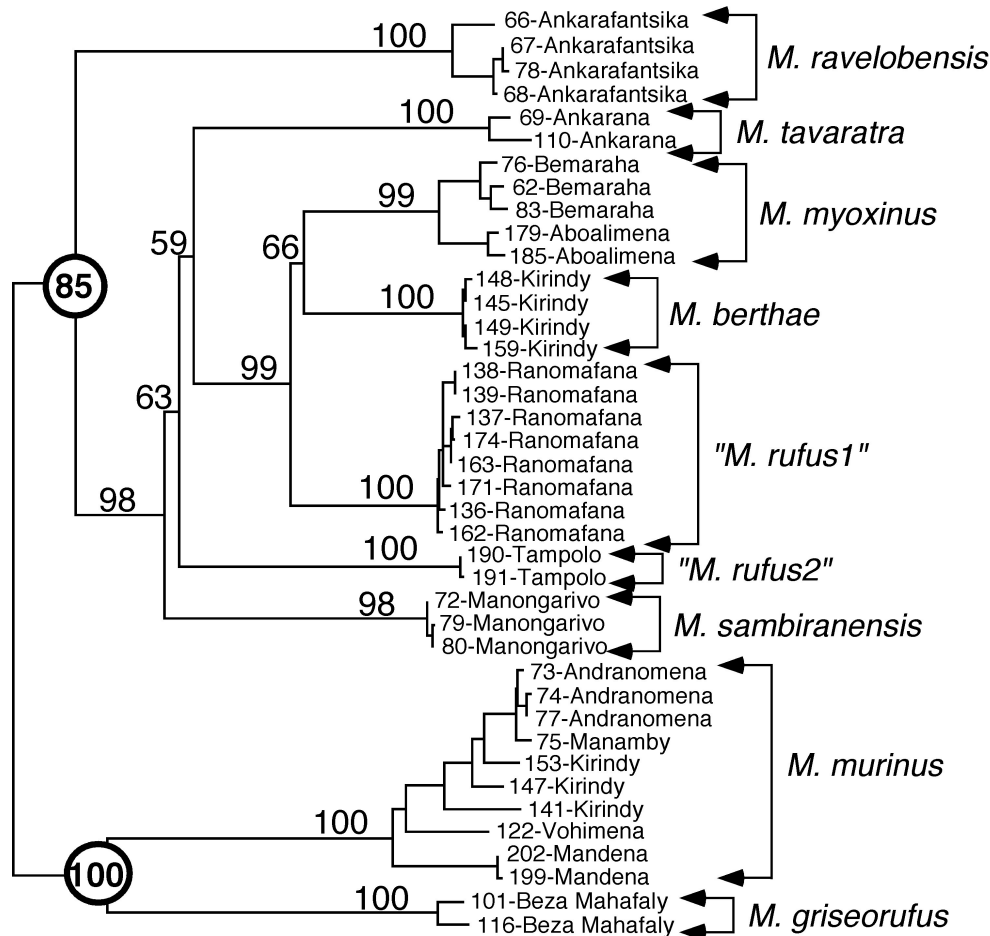


Figure 3:

Distance tree of 2404 bp of combined HV1, cytochrome *b* and COII. Tree was generated in PAUP* 4.0b4a (PPC) (Swofford, 1998) by using HKY85 correction and weighted least squares (power = 2) algorithm. Location of midpoint root was confirmed by multiple outgroup rootings. Numbers on branches indicate statistical support from 100 bootstrap replicates with one random addition per replicate. Circled numbers highlight bootstrap support for two primary clades.



Yoder (Fig. 3) ↑

Figure 4:

Phylogeographic model of mtDNA haplotypes that would be consistent with predicted east/west biogeographic disjunction.

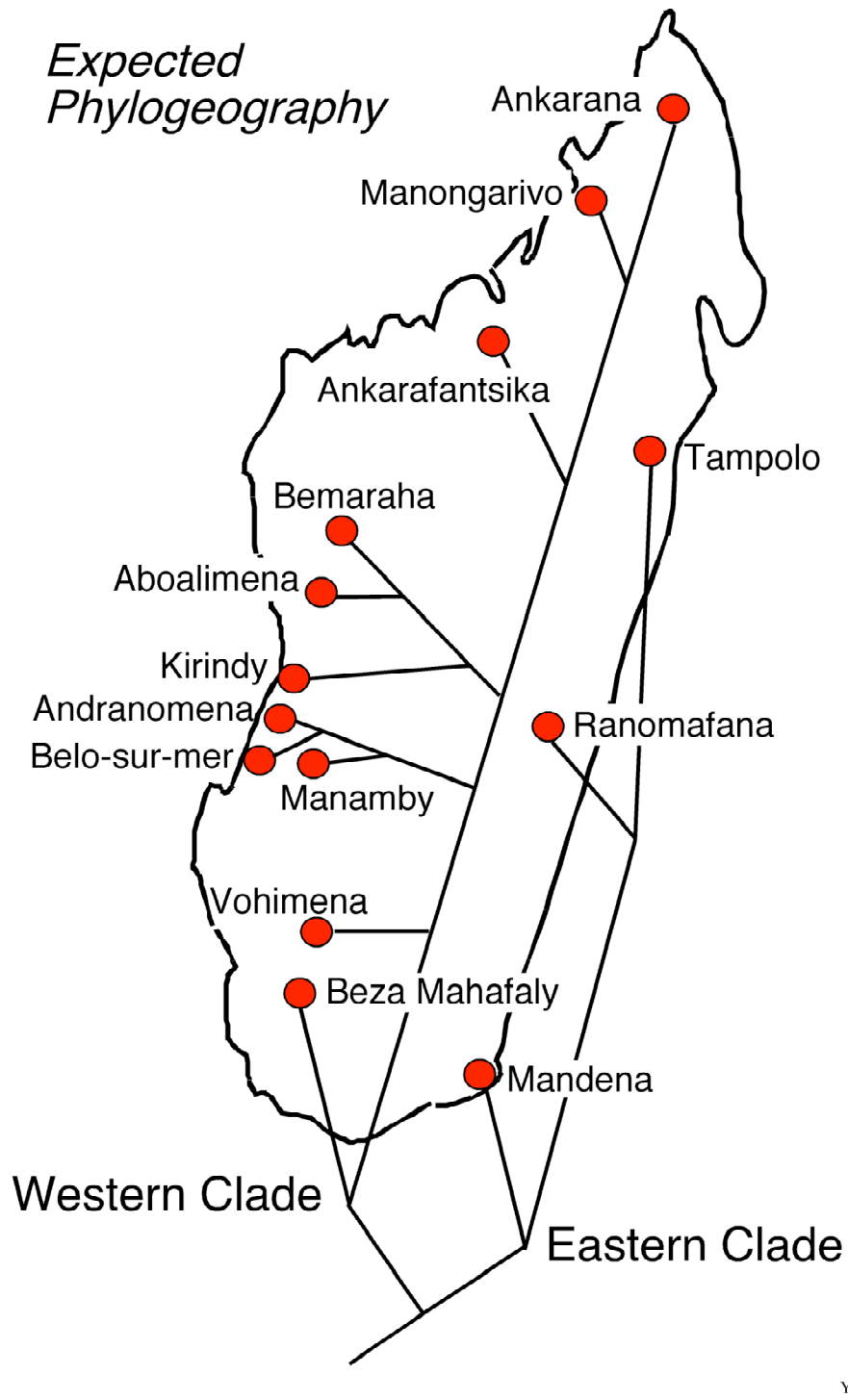
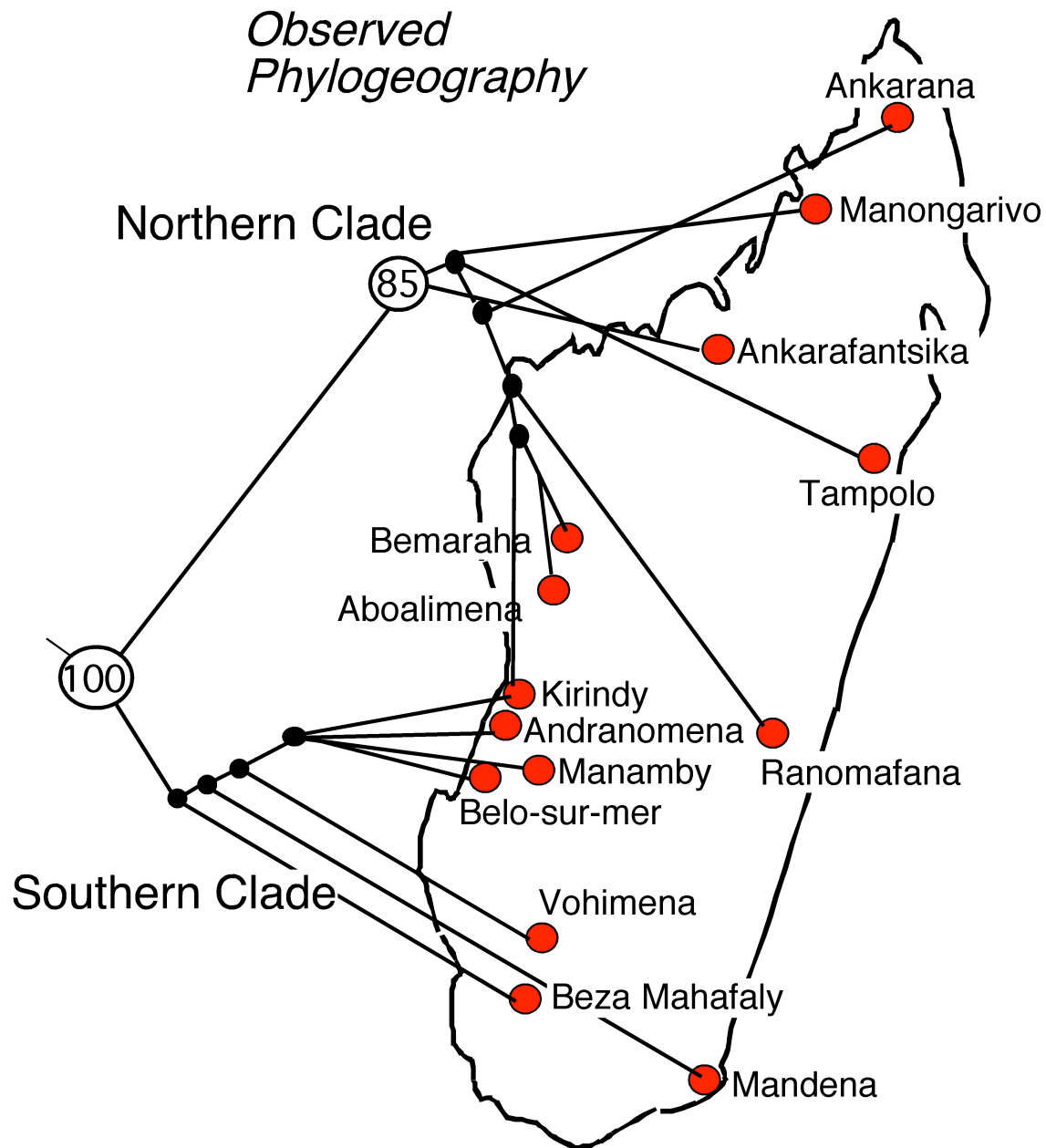


Figure 5:

Actual phylogeographic structure of mtDNA haplotypes sampled by the Yoder et al. (2000) study. Note strong statistical support for primary division into northern and southern clades. Pattern is subject to further testing with additional geographic and haplotype sampling.



Yoder (Fig. 5) ↑