

**Ecological separation of two sympatric species
of *Microcebus* spp. E. Geoffroy, 1812
in southern Madagascar**

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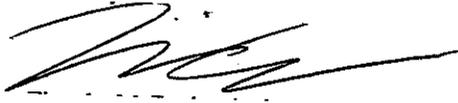
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To my family

20 June 2011

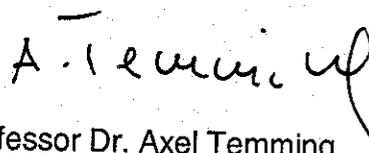
As a native speaker I hereby confirm that the doctoral thesis of S. Jacques Rakotondranary, titled "Ecological separation of two sympatric species of *Microcebus* spp. E. Geoffroy, 1812 in southern Madagascar" is written in good English grammar and comprehensible style.



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GENERAL INTRODUCTION

Ecologists have long been interested in the phenomena, mechanisms, and interactions that allow several species to live in the same area (Elton 1927, Gause 1934, Hutchinson 1959, Hardin 1960). Classical hypotheses suggest that two species cannot coexist if they share the same ecological niche (Elton 1927, Gause 1934). Observations of a number species sharing the same habitat suggest that species can coexist if they utilize different resources, adapt differently to dissimilar environmental conditions, or differ in size. Limited resources represent the actual basis for competition. Physiological, behavioural and morphological adaptations to abiotic conditions or size differences of the consumers represent traits that help to achieve partitioning of these limited resources (Smith and Smith 2001). These phenomena will be elaborated below.

1 Interspecific competition and niche partitioning in general

1.1 Resource partitioning

Resource partitioning occurs when two species utilize a certain resource differently due to behavioural, physiological or morphological variation. There are three major mechanisms that allow differential utilization of resources (Schoener 1974).

1.1.1 Temporal partitioning

Direct competition between sympatric species could be avoided if different species would use the same resource at different times of the day. This type of partitioning is possible, if the resource is not depleted by one of the species or it is quickly renewed (Gotelli *et al.* 1996). Temporal separation can be achieved on a daily basis, such as the separation of sympatric species of lemurs in Madagascar. Several species can use similar resources but are active either during the day or at night (Petter 1962; Ganzhorn 1989). On a longer, seasonal scale, sympatric gorillas and chimpanzees at Kahuzi-Biega National Park change their food composition seasonally. This has been interpreted as a way to reduce competition between these great apes (Yamagiwa 2006). Thus, time is one of the niche dimensions that can help to separate species on different scales (Case and Gilpin 1974; Carothers and Jaksic 1984; Albrecht 2001).

1.1.2 Spatial partitioning

Food and space are the major axes for niche partitioning (Schoener 1974). Spatial resource separation occurs when two competing species use the same resource by occupying different areas or habitats where the resource occurs. This can be the case at small scales (microhabitat differentiation; e.g., sympatric lemur species *Microcebus murinus* and *M. berthae* in western Madagascar show spatial separation on a local scale [Schwab and Ganzhorn 2004; Dammhahn and Kappeler 2008]; sympatric and possibly competing species of *Cheirogaleus major*, *C. medius* and *Microcebus murinus* in a littoral rain forest in south-eastern Madagascar differ in their vertical utilization of the habitat [Lahann 2008]). On a larger scale, ecologically similar species can occur in different geographical areas. These vicariant mouse lemurs represent different species that occupy the same niche in allopatry, such as probably different species of allopatric *Microcebus* spp. (Mittermeier et al., 2006) or various reptile species of Madagascar (Raxworthy and Nussbaum 1997).

1.1.3 Morphological differentiation

On a community level, competition over the same principle resources is thought to be reduced to levels that allow for coexistence if species differ in body mass by a factor of 2 and in length measurements by a factor of about 1.4 (Hutchinson 1959). This “Hutchinson’s rule” has been applied to explain body mass patterns in different animal communities, including primates (e.g., Ganzhorn 1999) as well as for sexual dimorphism in carnivores and herbivores (Dayan *et al.* 1989; Pérez-Barberia *et al.* 2008; from Rakotondranary 2011).

The classic example for more specific morphological differentiation or character displacement is represented by Darwin's finches on the Galapagos Islands (Lack 1947). Originating from rare (possibly single) colonization events, individuals were selected for different beak sizes, allowing several competing species of Darwin's finches to partition available resources. When food is scarce and the availability of specific seeds is reduced, such as during El Nino events, beak sizes can shift rather rapidly, illustrating rapid adaptations to fluctuating resource availabilities (Lack 1947, Grant 1999, Grant and Grant 2006).

1.2 Adaptations to environmental conditions

1.2.1 Different adaptations to environmental conditions

Species, possibly competing over the same resource, can differ in their abilities to use a resource under varying environmental conditions (abiotic and biotic). One species may be more competitive under one set of environmental circumstances, while another species is more competitive in another set of conditions. This is illustrated by fish that co-occur in intertidal salt marshes in coastal Virginia. These species use the same resource but differ in their tolerance of temperature, pH, and salinity (Dunson *et al.* 1993).

1.2.2 Life history traits

Another type of niche differentiation between sometimes is based on inter-specific trade-offs between life history traits that influence competition (e.g. fecundity, longevity) and those that allow species to reduce competition (e.g., dispersal) (Amarasekare 2003). The trade-off concept assigns species to two categories: (1) generalist species that are successful colonists with large geographic ranges and broad habitat specificity with high fecundity but sometimes rather short-lived and wide dispersal, can colonize free niches quickly as they arise. In contrast, (2) specialist species can be superior competitors, often characterized by low fecundity but high longevity. The trade-offs between reproduction and longevity have also been described with single lemur species living under different environmental conditions (Lahann *et al.* 2006; Lahann and Dausmann 2011).

1.3 Coexistence without niche differentiation

Competition between species is usually thought of in terms of two species interacting over limited resources (Krebs 2001). Thus, if populations of several different species do not reach the habitat capacity, coexistence may be possible. The same effect can be achieved when high predation pressure or parasitism keeps populations below carrying capacity. Examples are: (1) hispine beetles that live as adults in the rolled leaves of *Heliconia* plants. These very closely related beetle species eat the same food and occupy the same habitat but coexist without any evidence of segregation or exclusion (Strong 1982); (2) species-rich tadpole communities where species are not limited by resources. Rather, different species seem to converge and adapt to specific

environmental conditions, thus leading to the occupation of specific niches by multiple species (Strauss *et al.* 2010).

2 Interspecific competition and niche partitioning in primates

The concepts described above provide the framework for studies in evolution and community ecology of primates. Most studies focussed on differences in diet, spatial use of habitats (horizontal and vertical), and activity patterns as the major axes for niche partitioning. A global analysis on niche separation in sympatric species of primates showed that differences in spatial use of habitat and diet are the dominant forms of separation between potentially competing pairs of species (Schreier *et al.* 2009). More detailed case studies of sympatric primates showed that separation could be achieved by various combinations of differences in habitat utilization, food composition, or temporal separations (contr. to Fleagle *et al.* 1999).

3 Malagasy mouse lemurs

Mouse lemurs (*Microcebus* spp.) are the smallest of the Malagasy prosimians (body mass: 30-90 g; length: 23-29 cm [including tail]), nocturnal, solitary and omnivorous. They are present in a wide range of forests throughout Madagascar where suitable habitats remain, including primary, secondary and even disturbed forest habitats, with home ranges from one to two hectares (Martin 1972, Petter *et al.* 1977, Mittermeier *et al.* 2006).

Two species of the genus *Microcebus* coexist in various species combinations in several regions of Madagascar. In the western part of Madagascar, these species pairs include the widely distributed gray mouse lemur (*M. murinus*), ranging from littoral humid forest in the southeast to the northwest and other congeneric species with a locally restricted range, such as *M. ravelobensis*, *M. berthae* and *M. griseorufus* (Figure 1). Thus, these mouse lemurs provide excellent opportunities to investigate the mechanisms that allow species to coexist. Up to now studies of sympatric mouse lemurs illustrated various mechanisms that allow separation of species in the dry deciduous forest ecosystems. In western Madagascar, *M. murinus* and *M. berthae* differ in body mass by a factor of 2, are spatially separated, and differ in food composition (Schwab and Ganzhorn 2004; Dammhahn and Kappeler 2008, 2010). In northwestern Madagascar, *M. murinus* and *M. ravelobensis* show no size differences but are separated by large and small scale habitat utilization and food composition (e.g., Rakotondravony and Radespiel 2009; Thorén *et al.* 2011).

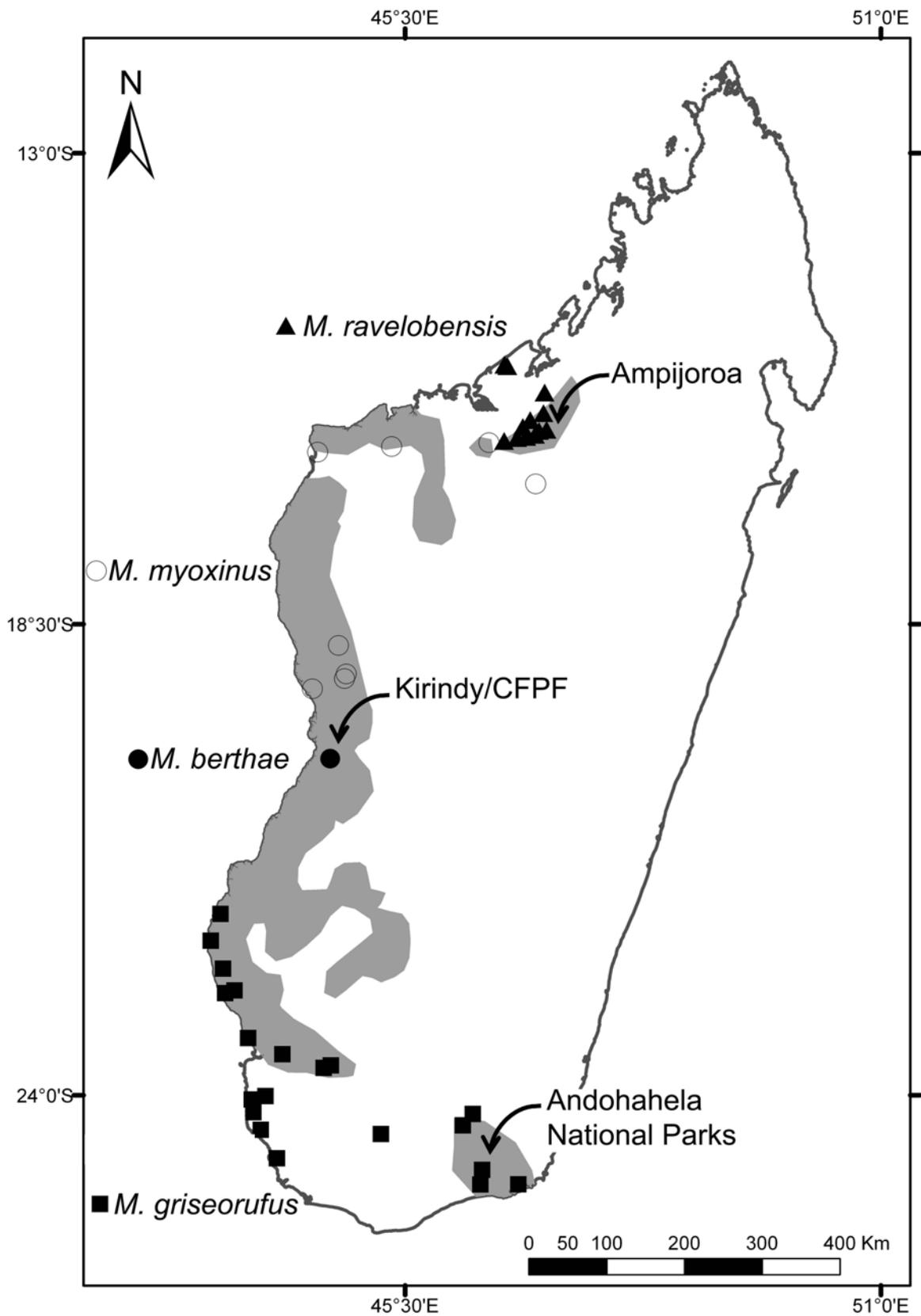


Figure 1. Distribution of *Microcebus murinus* (marked in gray) according to Mittermeier *et al.* (2006), *M. ravelobensis*, *M. berthae*, *M. griseorufus* and *M. myoxinus* (by courtesy of Lucienne Wilmé).

In this thesis, I focus on two species that co-occur in the dry spiny forest in southern Madagascar, the reddish-gray mouse lemur (*Microcebus griseorufus*) and the gray mouse lemur (*M. murinus*) (Figure 2). These two species are morphologically very similar and represent sister species (Yoder and Yang 2004), i.e., they are the least likely primate species to coexist due to their phylogenetic similarity (Houle 1997). The ecology, behaviour, genetics, and physiology of both species have been studied in the field and the laboratory (e.g., Ortmann *et al.* 1997, Schmid 2000, Yoder *et al.* 2002; Génin 2008, Kobbe and Dausmann 2009, Schmid and Ganzhorn 2009; Gligor *et al.* 2009). In areas of co-occurrence, separation of these two species was supposed to be achieved on the basis of habitat types. Where they occur in the same area, *Microcebus griseorufus* seems to be restricted to drier vegetation, such as dry spiny forest, while *M. murinus* occupies wetter sites, such as gallery forests (Beza Mahafaly: Rasoazanabary 2004; Berenty Speciale Reserve: Yoder *et al.* 2002). This separation is not consistent throughout the species' ranges. They can be found at the same spot and hybridize in some areas but maintain their morphological and genetic species identity at adjacent sites (Ganzhorn and Randriamanalina 2004, Gligor *et al.* 2009, Hapke *et al.* submitted). These findings raise the question of how *Microcebus murinus* and *M. griseorufus* achieve niche partitioning and maintain their species identities.



Figure 2. Gray mouse lemur (*Microcebus murinus*) (left) and reddish-gray mouse lemur (*Microcebus griseorufus*) (right).

I therefore examined the ecological separation of *Microcebus murinus* and *M. griseorufus* in southern Madagascar by investigating resource partitioning in diet and habitat utilization. My

study was carried out at Andohahela National Park in the extreme southeast of Madagascar. This area provides an ideal opportunity to investigate the various patterns and evolutionary constraints because it consists of a continuous environmental gradient ranging from evergreen humid rain forest to dry spiny forest. This gradient covers all types of forest ecosystems known from this portion of Madagascar (Andriaharimalala *et al.* in press; Goodman 1999) and therefore should allow both species to inhabit different types of vegetation and possibly show different forms of species interactions.

4. Aims of the study

The aims of this study were:

- (1) Investigate the environmental conditions that are associated with the distribution of the two *Microcebus* species and their hybrids (**Chapter 1**);
- (2) Study the possible mechanisms that allow the coexistence of these sympatric congeneric species (*M. murinus* and *M. griseorufus*) by investigating the potential separation with respect to food composition (**Chapter 2**). This study is based on stable isotopes as an indirect measure of the trophic level of the consumer and its prey;
- (3) Describe microhabitat utilization of both species (**Chapter 3**).

Before these aims could be pursued, criteria had to be developed and verified that would allow researchers to identify species unambiguously and to define individuals as hybrids. The morphological criteria are part of this thesis and described in Chapter 1. The genetic verification is presented separately (Hapke *et al.* submitted).

Specific questions addressed in the publications contributing to this thesis were:

Chapter 1

- (1) How are the different *Microcebus* species distributed along the environmental gradient in relation to environmental conditions and ecotones?
- (2) Does the morphology of a given species vary in relation to environmental conditions, reflecting the environmental/physiological constraints under different ambient conditions and possible adaptations on very small scales?
- (3) Is there evidence for morphological character displacement to reduce congeneric competition in sympatry vs. allopatry?

Chapter 2

(1) Do the isotope signatures of a given species vary in relation to environmental conditions?

(2) In allopatry, do the two species of *Microcebus* show seasonal changes in isotope signatures?

(3) In sympatry, do the two species of *Microcebus* show different isotope signatures indicating dietary niche separation?

(4) Do fecal analyses reflect possible dietary differences between species?

Chapter 3

(1) What are the characteristics of available habitats at sites where the species live in allopatry and at sites where species live sympatrically?

(2) Are there differences in microhabitat structures used by the species and their hybrids in sympatry?

(3) Are there indications for habitat selectivity by different *Microcebus* species?

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CHAPTER 1

Distribution and morphological variation of *Microcebus* spp. along an environmental gradient in southeastern Madagascar

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Abstract

The lemurs of Madagascar are known for their extraordinary levels of speciation. However, the mechanisms and environmental conditions that led to this diversity remain obscure. In this study we used three species of *Microcebus* (*M. griseorufus*, *M. murinus*, *M. rufus*) occurring along an environmental gradient as a model to investigate (1) how the different species are distributed in relation to variation in environmental conditions and ecotones; (2) whether or not the morphology of a given species varies in relation to environmental conditions; and (3) whether or not there is evidence for morphological character displacement to reduce congeneric competition in sympatry versus allopatry. The three species of *Microcebus* show clear associations with specific habitat types. Distributions overlap at ecotones. Nevertheless, the ecotone between dry spiny and gallery forest represents a species boundary between *Microcebus griseorufus* and *M. murinus* while the ecotone between dry spiny forest and evergreen humid forest represents the species boundary between *M. murinus* and *M. rufus*. Different ambient conditions are not reflected in changes in body measurements of *Microcebus murinus* living in different vegetation formations. There is no indication for character displacement in sympatry versus allopatry. Thus, differences in body mass or other morphological characteristics do not contribute to species separation between *Microcebus griseorufus* and *M. murinus*. The results confirm the importance of ecotones as species boundaries

as a condition postulated for the radiation of lemur and other species on Madagascar. They also demonstrate different habitat affinities of seemingly very similar lemur species and thus illustrate our very limited understanding of the actual selection pressures, adaptations of lemurs to their environments and their possible response to interspecific competition.

Keywords lemurs, primates, southeastern Madagascar, evolution, niche, competition

INTRODUCTION

Madagascar is known for its extraordinary biodiversity and high degree of microendemic species (Myers *et al.* 2000; Goodman and Benstead 2003). Lemurs are no exception. On average, their geographical ranges are orders of magnitudes smaller than the ranges of other primate species and the diversity of species exceeds the primate diversity in other areas of the world (Martin 1995; Mittermeier *et al.* 2008). The evolutionary processes that led to this extraordinary diversity remain enigmatic (Vences *et al.* 2009).

In other parts of the world, temperature and moisture are major factors that limit the distribution and abundance of plants and animals (Krebs 2009). In particular, the distributions of species are often confined by environmental discontinuities at ecotones (Terborgh 1971). Physical conditions (temperature) or biological resources can vary in parallel with environmental gradients. These adaptations may be reflected in intraspecific morphological variation in relation to environmental conditions (McNab 2010). Apart from adaptations to environmental conditions, interspecific competition may result in competitive exclusion or morphological or behavioral character displacements in sympatry versus allopatry (e.g., Grant and Grant 2006, 2010).

Several hypotheses have been formulated to explain the biogeographic and phylogeographic patterns of lemurs and the evolution of microendemics (e.g., Martin 1972; Richard and Dewar 1991; Rumpler 2000; Thalmann 2000; Pastorini *et al.* 2003; Goodman and Ganzhorn 2004a,b; Yoder *et al.* 2005; Ganzhorn *et al.* 2006; Masters *et al.* 2007; Groeneveld *et al.* 2009; Vences *et al.* 2009; Weisrock *et al.* 2010). In particular, two recent hypotheses aim to provide a general framework for the radiations of Madagascar's biota. Both emphasize the role of ecotones in parapatric or allopatric speciation. The "watershed hypothesis" postulates that river catchments

with sources at different altitudes produce sharp ecosystem boundaries during times of desiccation that lead to allopatric speciation (Wilmé *et al.* 2006). The “current climate hypothesis” postulates that species reach their distributional limits at ecotones created by climatic gradients (Dewar and Richard 2007). The two hypotheses thus imply the same mechanism but predict different patterns of microendemism and are supported by the distribution of different taxa (Pearson and Raxworthy 2009).

While evolutionary scenarios are of great interest, it remains an open question how species maintain their species identity once they have evolved into different forms. Understanding the mechanisms that maintain species identities is of interest as many of these taxa may hybridize under natural conditions in geographically restricted areas but remain morphological and genetically distinct over the rest of their range (Zaramody and Pastorini 2001; Thalmann *et al.* 2002; Vasey and Tattersall 2002; Wyner *et al.* 2002; Gligor *et al.* 2009). This is an issue not just for Madagascar but for other primate radiations (e.g., Merker *et al.* 2009; de Jong and Butynski 2011) and biology in general (Arnold and Martin 2010).

Allopatric and parapatric occurrences might reflect adaptations to specific habitat characteristics. These adaptations might also have come about by interspecific competitive displacement, as interspecific competition is supposed to be aggravated between congeneric species occurring in sympatry (Houlé 1997). Compared to other regions of the world, Madagascar seems particularly rich in the sympatric occurrence of congeneric species that seem to have rather similar habitat and food requirements, such as sympatric species of *Microcebus*, *Cheirogaleus*, *Hapalemur* and *Eulemur* (Blanco *et al.* 2009; Wright 1999). On a community level, competition over the same principle resources is thought to be reduced to levels that allow coexistence if species differ in body mass by a factor of 2 and in length measurements by a factor of about 1.4 (Hutchinson 1959). This “Hutchinson’s rule” has been applied to explain body mass patterns in different animal communities, including primates (e.g., Ganzhorn 1999) as well as for sexual dimorphism in carnivores and herbivores (Dayan *et al.* 1989; Pérez-Barberia *et al.* 2008).

Among the lemurs of Madagascar, the genus *Microcebus* has been the subject of numerous phylogeographic studies (Yoder *et al.* 2000; Radespiel *et al.* 2008; Louis *et al.* 2008; Weisrock *et al.* 2010). These studies describe sympatric species pairs (*Microcebus murinus* and *M. berthae*) that match Hutchinson’s rule, and also niche differentiation between these and other sympatric

congeneric species or species turnover at ecotones (Yoder *et al.* 2002; Rendigs *et al.* 2003; Schwab and Ganzhorn 2004; Radespiel *et al.* 2006; Dammhahn and Kappeler 2008; Génin 2008). Species turnover at ecotones is of particular interest for species that have diverged rather recently, such as the sister species *Microcebus griseorufus* and *M. murinus* (Yoder and Yang 2004) which occur sympatrically in southern Madagascar. Where they occur in sympatry, *Microcebus griseorufus* seems to be restricted to drier parts of the habitat, such as dry spiny forest, while *M. murinus* occupies wetter sites, such as gallery forests (Yoder *et al.* 2002). In the case of allopatry, *Microcebus griseorufus* occupies a variety of vegetational types including gallery forests and various forms of dry spiny forests (Yoder *et al.* 2002; Génin 2008; Rakotondranary *et al.* 2010). In the evergreen humid forests, *Microcebus murinus* is replaced by *M. rufus*. *Microcebus rufus* evolved within a lineage that split off from the *M. griseorufus* and *M. murinus* group about 5 - 12 million years ago; i.e. much earlier than the divergence between *M. griseorufus* and *M. murinus* (Yoder and Yang 2004). So far, there are no reports of hybridization between *Microcebus rufus* and *M. murinus*, while hybrids are known between *M. murinus* and *M. griseorufus* (Gligor *et al.* 2009). An investigation of the mechanisms that either maintain or generate the distinct identities of these closely-related taxa may help to understand the mechanisms postulated to underlie species radiations in Madagascar (Wilmé *et al.* 2006; Pearson and Raxworthy 2009; Vences *et al.* 2009).

Andohahela National Park in the extreme south-east of Madagascar provides an ideal opportunity to investigate the various patterns and evolutionary constraints along a continuous environmental gradient ranging from evergreen humid rainforest to dry spiny forest. This gradient covers all types of forest ecosystems known from this portion of Madagascar (Goodman 1999; Andriaharimalala *et al.* in press). Previous studies have revealed a clear separation of *Microcebus* spp. between humid rain forest (*Microcebus rufus*) in the east and dry spiny forest in the west of Andohahela (*M. cf. murinus*; Feistner and Schmid 1999). At the time of these earlier studies, the different forms of *Microcebus* of the dry forest ecosystems had not been recognized as distinct species. Our own subsequent studies indicated that the dry ecosystems of the western part of Andohahela contained *Microcebus murinus* and *M. griseorufus* occurring in allopatry or sympatrically with some forms showing intermediate phenotypes (Rakotondranary *et al.* unpubl.).

Vegetation in Andohahela forest is continuous along the environmental gradient and all species have the opportunity to move across the different habitats. Thus the distribution of species is

likely to reflect their preferred environmental conditions in concert with possible interspecific competition.

As a basis for further studies on speciation, physiological and ecological niche differentiation under the impact of congeneric competition, we addressed the following questions:

1. How are the different *Microcebus* spp. distributed along the environmental gradient in relation to environmental conditions and ecotones?
2. Can the two sister species *Microcebus griseorufus* and *M. murinus* be distinguished based on external morphological characteristics?
3. Does the morphology of a given species vary in relation to environmental conditions, reflecting the environmental/physiological constraints under different ambient conditions and reflecting possible adaptations on very small scales?
4. Is there evidence for morphological character displacement to reduce congeneric competition in sympatry versus allopatry? If differences in body mass or other morphological measures reflect a mechanism to reduce competition over limited resources between the *Microcebus* spp. of Andohahela, we predict that *Microcebus griseorufus* and *M. murinus* would differ more in their morphological measures at sites where they live in sympatry than at sites where only one of the species occurs.

METHODS

Study Sites

The study took place in the Andohahela National Park (Parcel 1, Parcel 2 and between Parcel 1 and 2) in southeastern Madagascar. The park is characterized by a steep rainfall gradient from 400 mm per year in the west to 2400 mm per year in the east with contiguous vegetation formations ranging from dry spiny forest (Parcel 2) to evergreen humid forest (Parcel 1) (Barthlott *et al.* 1996; Goodman 1999). Based on the vegetation classification of Moat and Smith (2007) we selected seven different sites in three types of vegetation (rainforest, gallery forest, dry spiny forest) along two transects from the humid east to the dry west. The northern transect consisted of sites at Mahamavo, Ankoba, Ambatoabo and Hazofotsy and the southern transect was composed

of sites at Ebosika, Tsimelahy and Mangatsiaka (see electronic supplement S1; Fig. 1). Andriaharimalala *et al.* (in press) verified the classification of the different sites by detailed vegetation studies. We georeferenced the sites with a GPS and extracted the altitude of the site from Google Earth version 5.2.1.1588.

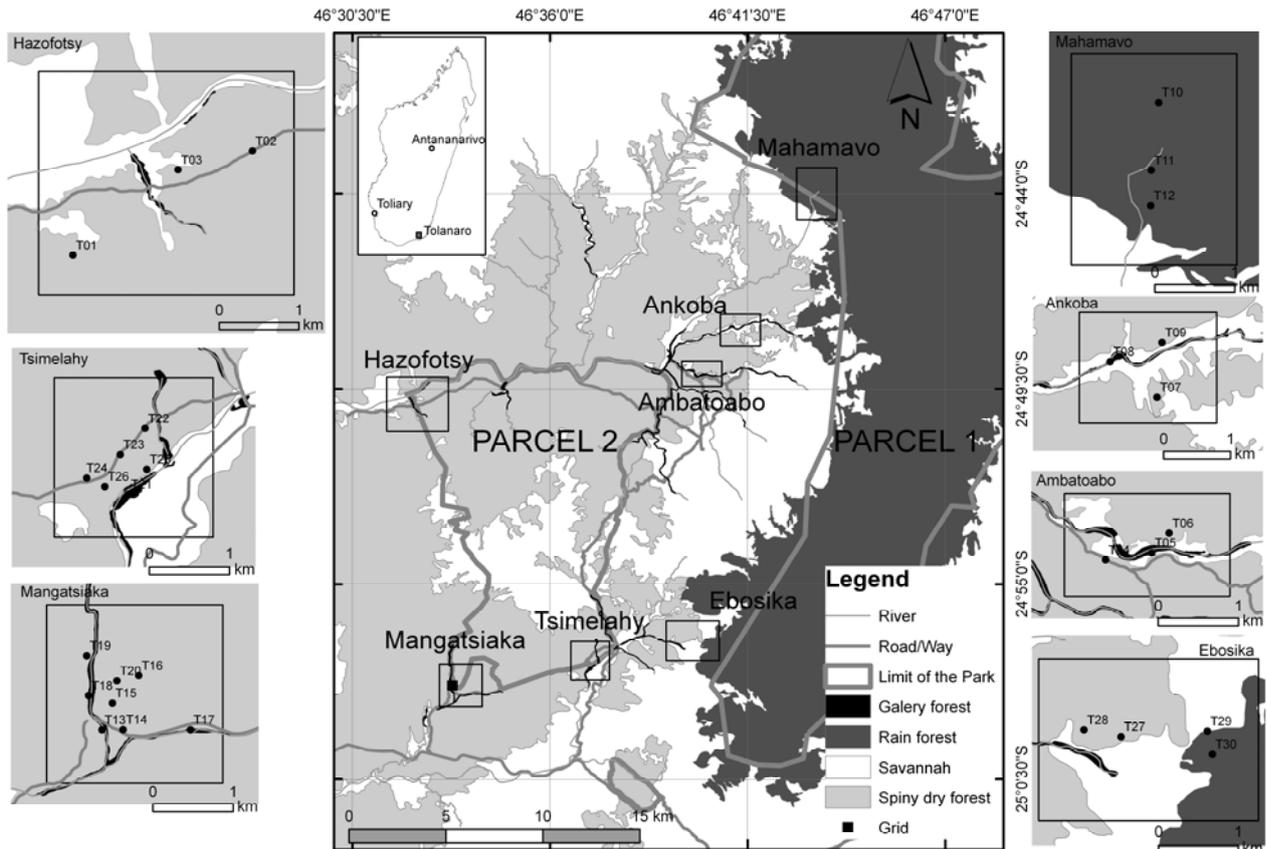


Fig. 1 Study sites in and between Parcels 1 and Parcel 2 of Andohahela National Park (modified from Google Earth).

We measured air temperature (in °C) and relative humidity (in percent) in the different habitat types with 11 data loggers (Hygrochron IButton/DS1923, Dallas Semiconductor, USA) placed in shaded places. We programmed loggers to record data every two hours. Temperature measurements started in October 2006, September 2007, and May 2008 in the gallery forest, dry spiny forest and humid forest, respectively (Fig. 2). Humidity measurements started in October 2006, September 2007, and November 2008 in the gallery, dry spiny and humid forest, respectively (Fig. 3).

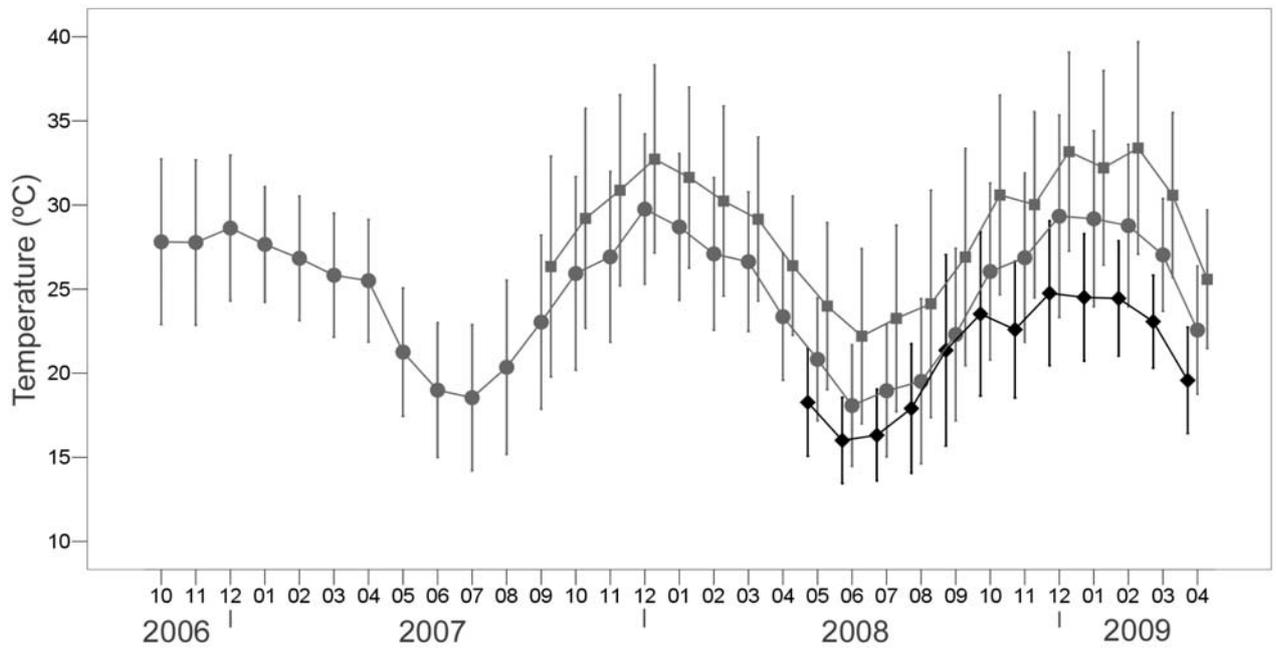


Fig. 2 Monthly temperature in different vegetation formations in Andohahela (◆rain forest, ■dry spiny forest, ●gallery forest). Values are means and standard deviations.

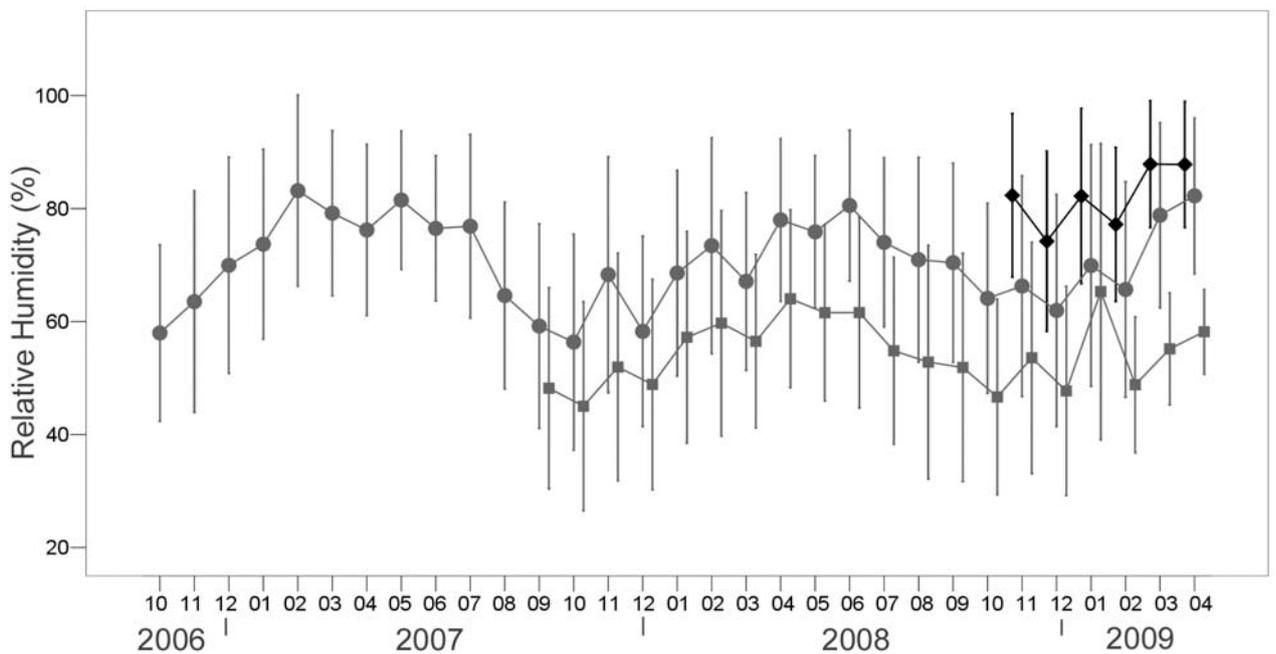


Fig. 3 Monthly relative humidity in different vegetation formations in Andohahela (◆rain forest, ■dry spiny forest, ●gallery forest). Values are means and standard deviations.

Study Period

From September 2006 to June 2009 we captured *Microcebus* spp. at the different sites during the end of the dry season (September, October, November) and after the birth season towards the end of the wet season (April, May, June).

Trapping

We captured animals at 30 trap lines of 475 m length within the three vegetation formations (see electronic supplement S1; Fig. 1). Each of the 30 transects was composed of 20 trapping sites, spaced at 25 m intervals. At each site, we installed two Sherman traps ($7.7 \times 7.7 \times 23$ cm) in a tree. In addition, we established a rectangular trapping grid at Mangatsiaka with 200 trapping stations (10 x 20 stations spaced at 25 m intervals), each consisting of two Sherman traps. The coordinates of the southwest corner of the trapping grid were ($24^{\circ} 57' 52.27''$ S, $46^{\circ} 33' 17.25''$ E). We added body mass and morphological measurements of the individuals caught in this grid to the samples of the trap lines. We baited traps with bananas for four successive nights per transect and checked the traps early in the morning. We anesthetized captured *Microcebus* with 0.01 - 0.03 ml of Ketanest [100 mg/ml].

We marked each mouse lemur individually using either coded ear clipping or a subcutaneous transponder (Trovan® Passive Transponder System, Typ ID-100). We kept the mouse lemurs in traps in the shade to recover from anesthesia and provided them with banana and water. We released the animals at their trapping site at dusk of the same day they were trapped. We identified recaptured animals based on the transponder and released them immediately.

Species Characterization

In the field, we identified species according to morphological measurements. Since it was unclear whether species identification based on phenotypes was reliable, A. Hapke verified the identifications by mtDNA and microsatellite analyses (Hapke et al. pers. comm.). A. Hapke used ear tissue samples stored in 90% ethanol for the genetic analyses. Except for *Microcebus rufus* (from the humid forest) that could be distinguished easily from all other forms, he genotyped all individuals considered in the present publication at one mitochondrial locus (the hypervariable

region 1 (HV1) of the control region) and at nine nuclear microsatellite loci. The genetic analyses and the procedures to determine hybrids and reputed backcrosses follow Gligor *et al.* (2009). The genetic analyses are presented elsewhere (Hapke *et al.* pers. comm.). They confirmed our field classification.

Microcebus spp. reproduce between December and March during the wet season (Wright 1999). Thus, animals caught between April and June could be either juveniles or adults. As *Microcebus* reaches sexual maturity in their first year of life, we considered all animals caught after the hibernation period between September and November to be sexually mature. Based on this assumption, we used the lowest body mass of an individual caught between September and November as the threshold to distinguish juveniles from adults. Since we did not capture any *Microcebus rufus* between September and November, we based our classification of juveniles on the minimum body mass of individuals trapped by Blanco (2008) in the humid forest of Ranomafana in October. Based on these criteria, we considered individuals as adults at a body mass of 47 g for *Microcebus murinus*, 42 g for *M. griseorufus*, 48 g for hybrids (*M. griseorufus* x *M. murinus*) and 34 g for *M. rufus*.

S. J. Rakotondrany took morphological measurements on the anaesthetized animal. Here, we present only measurements from adults. Tibia length represents the greatest length of the tibia. Other measurements were made according to Rasoloarison *et al.* (2000). All longitudinal measurements were taken to the nearest mm:

Ear length: from the notch at the base of the ear to the distalmost edge of the pinna.

Head and body length: from the tip of the nose to the distalmost point of the body (at base of tail).

Hindfoot length: from the back edge of the heel to the tip of the longest toe (not including claw).

Tail length: from the base of the tail (at right angles to the body) to the end of the distalmost vertebra, excluding terminal hair tufts.

Mass: measured with Pesola spring scales to the nearest g.

The following measurements follow Hafen *et al.* (1998):

Head length: distance between the nostrils and the foramen magnum.

Head width: bizygomatic distance perpendicular to head length.

Ear width maximum width measured perpendicular to ear length.

Statistical Analysis

The morphological data do not deviate from normality according to Kolmogorov-Smirnov-tests. We used a principal components analysis (PCA) on head and body length, tail length, head length, head width, ear length, ear width, tibia length and hindfoot length (see electronic supplement S3) to derive a measure of the overall shape of the animals, extracting all eigenvalues >1 .

We used parametric analysis of variance (ANOVA) and Scheffé's post-hoc tests for the comparisons of morphological measures between species and hybrids. We used t-tests for pairwise comparisons. Data were analyzed with SPSS 13.0. We used only morphometric measurements recorded at the first capture of each animal, regardless of any subsequent recaptures. To compare species (*Microcebus griseorufus*, *M. murinus*, *M. rufus*), we added data from an additional nine individuals captured outside the transect work but in the immediate vicinity of the transects and in the same vegetation formation. We considered differences significant at $p \leq 0.05$.

RESULTS

Abiotic factors: Temperature and relative humidity

Figures 2 and 3 illustrate monthly temperatures and humidity in the three types of vegetation in Andohahela. The dry spiny forest is characterised by the highest and the humid rain forest by the lowest temperatures. December and January are the hottest months in all vegetation types, while June and July are the coldest. Relative humidity is highest in the rain forest, followed by gallery and dry spiny forest. On a site-specific level, the principal differences remain the same, but similar vegetation formations show additional variation between sites located in the northern transect (Mahamavo – Ankoba – Ambatoabo – Hazofotsy) and in the southern transect (Ebosika – Tsimelahy – Mangatsiaka). Measurements taken during the hottest (December, January) and coldest months of the year (June, July) indicate that the rainforest, gallery and dry spiny forests (except for Hazofotsy) have higher temperatures and lower humidity in the southern than in the northern transect. The dry spiny forest of Hazofotsy does not follow this pattern, but this dry vegetation formation is likely to represent a special form of dry spiny forest that is not matched by

a site in the southern transect (see electronic supplement S2; Fig. 2, 3). Daily fluctuations in temperature and humidity increase in the drier habitats with less vegetation cover (Fig. 4, 5).

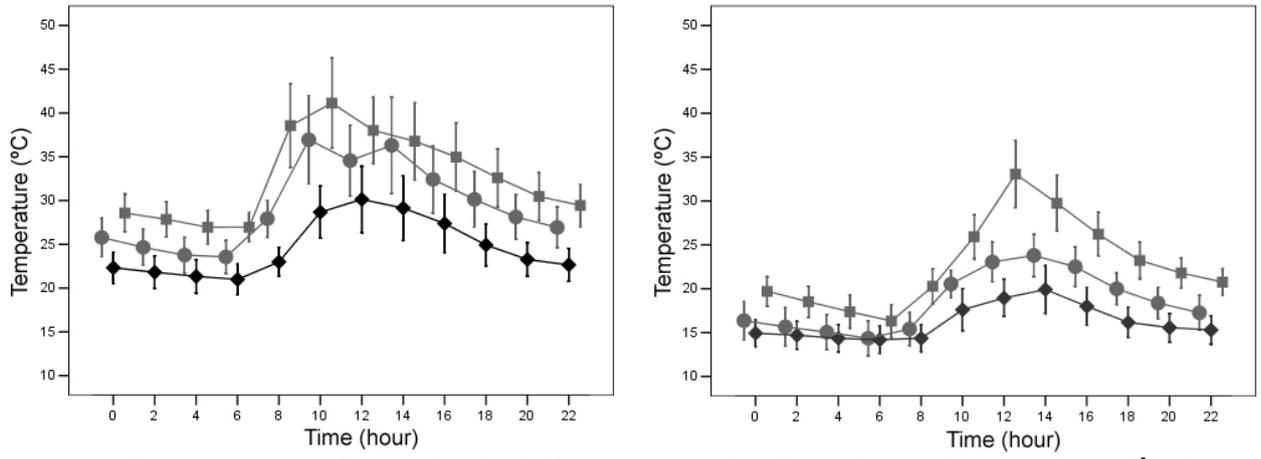


Fig. 4 Daily temperature fluctuation in different vegetation formations of Andohahela (◆rain forest, ■dry spiny forest, ●gallery forest). Values are means and standard deviations of two hour intervals in the hot season (December 2008, January 2009; left) and the cold season (June, July 2008; right).

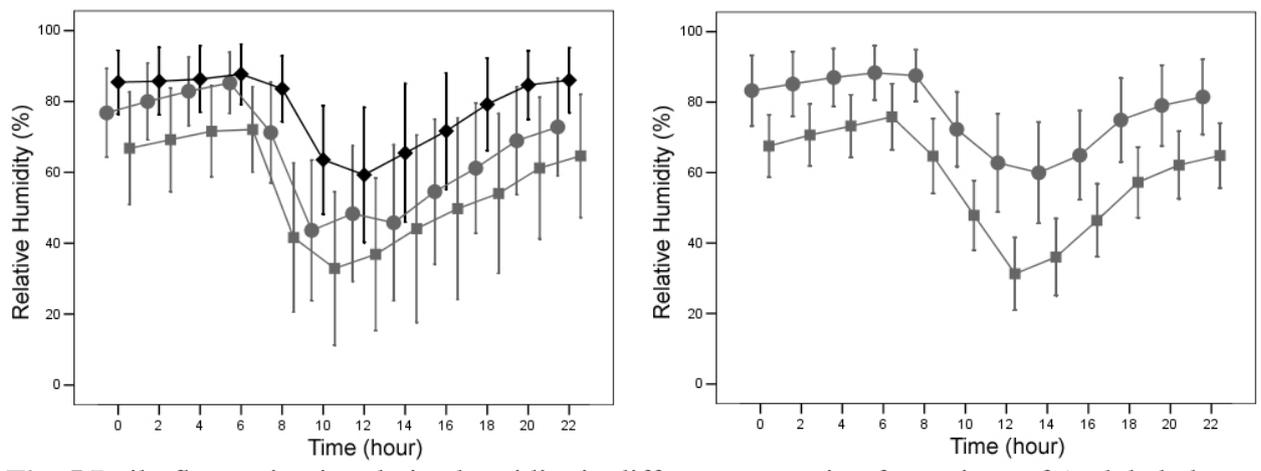


Fig. 5 Daily fluctuation in relative humidity in different vegetation formations of Andohahela (◆rain forest, ■dry spiny forest, ●gallery forest). Values are means and standard deviations of two hour intervals in the hot season (December 2008, January 2009; left) and the cold season (June, July 2008; right).

Distribution of *Microcebus* spp. along the environmental gradient

We captured 163 different individual *Microcebus* spp. in 7040 trap-nights at the 30 standardized trap lines along the transect (44 capture sessions with 40 traps per trap line, set for four nights; Table I). We found *Microcebus rufus* only in the rainforest of Mahamavo, the site with the lowest temperatures and highest humidity (see electronic supplement S2). We caught *Microcebus griseorufus* only in the dry spiny forest, but trapped *M. murinus* in all three vegetation types. We captured animals classified genetically as hybrids between *Microcebus griseorufus* and *M. murinus* in transects and the trapping grid where dry spiny forest and gallery forest were in close proximity. Thus, despite the occurrence of some hybrids, the limits of all *Microcebus* spp. were fairly well defined by ecotones.

Seasonal and intraspecific variation in body mass and morphology

We classified 139 of the 163 animals caught in transects and nine individuals caught in the grid at Mangatsiaka as adults based on our body mass criteria (Fig. 1; Table I; see electronic supplement S1). Seasonal differences in body mass were pronounced in *Microcebus murinus*. Male body mass was lower at the end of the wet season (54.0 ± 4.4 g, $N = 27$) than at the end of the dry season: (61.6 ± 6.0 g; $N = 14$; $t = 4.56$, $p < 0.001$). However, none of the other morphological measurements differed between seasons, so we did not consider the seasonal effects separately in subsequent analyses.

Of the three species, only females and males of *Microcebus murinus* differed in body mass, with females being heavier (65.0 ± 11.9 g; $N = 41$) than males (56.6 ± 6.2 g; $N = 41$; $t = 4.04$, $p < 0.001$). With respect to morphological measurements, sex differences were only apparent in head and body length and ear width (see electronic supplement S3). Females of *Microcebus murinus* and *M. griseorufus* have larger head and body length than males (*M. murinus*: females: 14.3 ± 1.1 cm, $N = 40$; males: 13.7 ± 0.6 cm, $N = 41$; $t = 2.93$, $p < 0.01$; *M. griseorufus*: females: 14.0 ± 1.1 cm, $N = 22$; males: 13.0 ± 1.0 cm, $N = 14$; $t = 2.64$, $p < 0.05$). *Microcebus murinus* males have wider ears (1.83 ± 0.11 cm; $N = 41$) than females (1.77 ± 0.11 cm; $N = 41$; $t = 2.65$, $p < 0.01$). None of the other measures differed between the sexes (see electronic supplement S3).

Table I Numbers of *Microcebus* captured per four-night capture session at each transect. The blank cells indicate that no capture was performed in a given season. Hybrids represent crosses or backcrosses between *M. griseorufus* and *M. murinus* as characterized by microsatellites (Hapke unpubl.).

Site	Transect	Year	September-October-November				April-May-June				
			<i>M. griseorufus</i>	Hybrid	<i>M. murinus</i>	<i>M. rufus</i>	Year	<i>M. griseorufus</i>	Hybrid	<i>M. murinus</i>	<i>M. rufus</i>
Hazofotsy	T01	2007	11	0	0	0	2008	2	0	0	0
	T02	2007	2	1	0	0					
	T03						2008	0	0	0	0
Ambatoabo	T04	2007	0	0	3	0	2008	0	0	1	0
	T05	2007	0	1	6	0	2008	0	0	2	0
	T06						2008	0	0	6	0
Ankoba	T07	2007	0	0	0	0	2008	0	0	1	0
	T08	2007	0	0	0	0					
	T09	2008	0	0	0	0	2008	0	0	4	0
Mahamavo	T10	2007	0	0	0	0					
	T11						2008	0	0	0	6
	T12						2008	0	0	0	12
Mangatsiaka	T13	2006	0	1	0	0					
	T14	2006	1	1	0	0					
	T15	2006	0	0	1	0	2008	3	1	0	0
		2007	2	0	1	0					
	T16						2008	0	1	2	0
	T17	2006	0	0	2	0	2008	0	0	6	0
		2007	0	2	2	0					
	T18	2007	0	0	6	0	2008	0	1	12	0
	T19						2008	1	2	6	0
	T20						2008	1	3	3	0
Tsimelahy	T21	2006	0	0	5	0	2008	0	0	1	0
	T22	2006	0	0	4	0	2008	0	0	3	0
	T23	2006	3	0	0	0					
	T24	2006	7	1	0	0	2008	3	0	0	0
	T25	2006	0	0	4	0	2008	0	0	1	0
							2008	3	0	1	0
Ebosika	T27	2006	0	0	2	0					
	T28	2006	1	1	0	0					
	T29	2006	0	0	0	0					
	T30	2006	0	1	3	0					
Total			27	9	39	0		13	8	49	18

Intraspecific morphological variation in relation to environmental conditions

We found no significant difference in the morphology of populations inhabiting the different vegetation formations. Females from the rainforest had very high body mass, but our sample size is too small to conclude that females have higher body mass in the humid forest than in the gallery and dry spiny forest (Table II).

Interspecific differences in body mass and morphology

Microcebus rufus was clearly distinct from *M. murinus* and *M. griseorufus* based on body mass, head body length, tail length, ear measurements, the length of the tibia (Table III). *Microcebus griseorufus* had longer tails than *M. murinus*. Though these two species did not differ significantly in any other morphological measurement, *Microcebus griseorufus* leaves the impression of being smaller than *M. murinus* with a smaller and more slender head. When combining these head measurements with the tail length, the ratios of tail length to head length and of tail length to head width provided very clear criteria separating the two species. Based on single morphological characteristics, hybrids resemble *Microcebus murinus* more than *M. griseorufus* in these traits (Table III; Fig. 6). A principal component analysis yielded three principle components (PCs) (see electronic supplement S4). The first reflects increasing size, the second reflects large heads, and the third represents animals with short tails and wide heads. The first two PCs did not separate the different taxa, but *Microcebus griseorufus* and *M. murinus* were clearly distinct for PC3 and the hybrids were intermediate between the two (Table III; see electronic supplement S4).

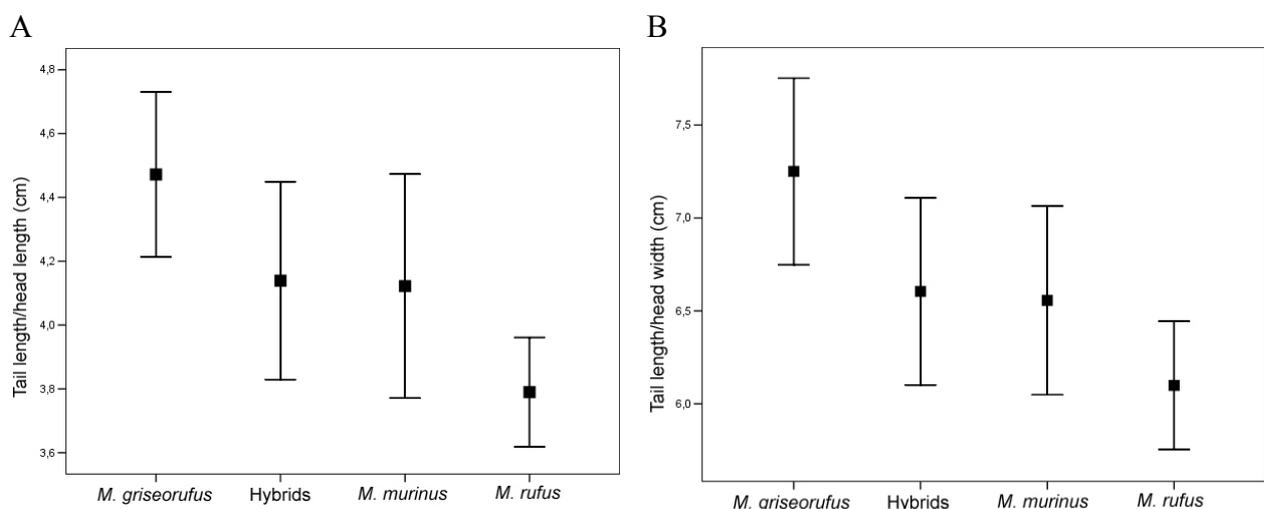


Fig. 6 Ratios of tail length/head length (A) and tail length/head width (B) of *Microcebus*. Values are means and standard deviations.

Effects of possible interspecific competition on morphology

Microcebus griseorufus males were smaller with lower body mass and smaller head and body length when in sympatry with *M. murinus* than when in allopatry (Table IV). None of the other measurements indicated any difference between sympatry and allopatry, neither for *Microcebus griseorufus* nor for *M. murinus*.

DISCUSSION

The ultimate goal of the study was to contribute to the understanding of the processes that led to the evolution of microendemic lemur species and the mechanisms that allow sympatric congeneric species to coexist. The region represented by the Andohahela National Park is ecologically extremely complex and had a very dynamic recent history that might represent a model for Madagascar's evolutionary history. Excavations of subfossil remains at the cave of Andrahomana a few kilometres south of Andohahela suggest that the western slopes of Andohahela have been subject to various episodes of climate changes with waxing and waning of the different ecosystems (Burney *et al.* 2004, 2008). Within the framework of these complex changes, the situation in Andohahela has components of the watershed as well as of the current climate scenario. The ecotone between the humid evergreen forest and the dry spiny forest corresponds to the current climate hypothesis where the evergreen humid forest represents the distributional limit for *Microcebus rufus*. In contrast, the distributions of *Microcebus murinus* and *M. griseorufus* resemble the watershed scenario. According to this scenario, species could retreat into mesic habitats at times of dessication. These mesic refugia are represented by watersheds that extend from the evergreen humid forest. *Microcebus murinus* could be interpreted as such a species that is confined by the watershed. *Microcebus griseorufus* would then represent a species that has adapted to the dry conditions outside the watershed.

The distribution of *Microcebus* spp. along the environmental gradient matches the pattern found at other sites. *Microcebus murinus* is the most widespread species on Madagascar. However, along the environmental gradient represented in our study site, it is replaced by *Microcebus griseorufus* in the driest part of the study area at Hazofotsy and by *M. rufus* in the humid forest of Mahavavo.

Table II Comparison of adult *Microcebus murinus* in the different forest types. body mass in gram; all other morphological measures in cm.

	Rain forest				Gallery forest				Dry spiny forest				ANOVA	
	N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	F	p
Body mass (female)	2	73.0	87.0	80.0±9.9	15	47.0	83.0	65.1±10.2	24	47.0	90.0	63.8±12.6	1.781	0.182
Body mass (male)	1			59.0	18	47.0	70.0	56.5±7.2	22	47.0	69.0	56.5±5.5	0.075	0.928
Head and body length (female)	2	13.0	15.0	13.9±0.9	14	11.0	16.0	14.4±1.3	24	12.0	16.0	14.2±0.9	0.238	0.790
Head and body length (male)	1			13.0	18	13.0	15.0	13.7±0.7	22	13.0	15.0	13.7±0.6	0.669	0.518
Tail length	3	14.4	15.0	14.6±0.3	32	13.0	16.6	14.5±0.9	47	12.0	16.7	14.6±1.0	0.150	0.861
Head length	3	3.5	3.9	3.7±0.2	33	2.5	3.9	3.5±0.2	47	2.5	3.9	3.5±0.2	1.494	0.231
Head width	3	2.0	2.4	2.2±0.2	33	2.0	2.8	2.2±0.1	46	2.0	2.8	2.2±0.1	0.114	0.893
Ear length	3	2.1	2.4	2.3±0.2	33	1.4	2.8	2.4±0.2	47	2.1	2.7	2.4±0.2	0.668	0.515
Ear width	3	1.7	1.8	1.8±0.1	33	1.5	2.1	1.8±0.1	47	1.6	2.0	1.8±0.1	0.359	0.700
Tibia length	3	4.0	4.2	4.1±0.1	31	3.7	4.5	4.2±0.2	46	3.8	4.5	4.1±0.2	0.812	0.448
Hindfoot length	3	3.5	3.5	3.5±0.1	33	3.0	3.6	3.3±0.1	47	3.1	3.6	3.4±0.1	2.528	0.086
Tail length/Head length	3	3.8	4.1	3.9±0.2	32	3.7	5.8	4.1±0.4	47	3.4	5.8	4.1±0.4	0.448	0.641
Tail length/Head width	3	6.1	7.3	6.6±0.6	32	4.8	7.3	6.5±0.6	46	5.2	7.5	6.6±0.5	0.133	0.875

N = sample size; Min = minimum, Max = maximum, mean ± standard deviations. F-values indicate differences between characters according to ANOVA

Table III Body mass and morphological measurements of *Microcebus* spp.; body mass in gram; all other morphological measures in cm; variables with significant differences in bold. “Head width” differences are not significant according to Scheffé’s post-hoc test. PC1 - PC3: Principal components based on morphological measurements; *M. rufus* is not included in the calculation of these PCs.

	<i>M. griseorufus</i>				<i>M. murinus</i>				Hybrids (<i>Mg</i> x <i>Mm</i>)				<i>M. rufus</i>				ANOVA	
	N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	F	p
Body mass (female)	22	42.0	72.0	52.9±7.4^{ab}	41	47.0	90.0	65.0±11.9^a	6	48.0	58.0	53.7±3.6^{ab}	9	35.9	72.9	46.7±12.8^b	11.55	0.001
Body mass (male)	14	42.0	60.0	52.2±4.6^a	41	47.0	70.0	56.6±6.2^a	6	50.0	65.0	56.7±5.7^a	8	37.9	56.9	43.3±6.3^b	12.43	0.001
Head and body length (female)	22	12.0	16.0	14.0±1.1^a	40	11.0	16.0	14.3±1.1^a	6	12.0	14.0	13.5±0.9^{ab}	9	12.9	13.9	12.5±0.5^b	7.542	0.001
Head and body length (male)	14	10.0	15.0	13.0±1.0^{ab}	41	13.0	15.0	13.7±0.6^a	6	11.0	15.0	13.4±1.3^{ab}	8	12.9	14.9	12.5±0.6^b	6.574	0.001
Tail length	36	13.7	17.0	15.5±0.9^a	82	12.0	16.7	14.6±0.9^b	12	12.5	16.0	14.5±1.1^b	17	12.0	15.0	13.2±0.8^c	25.459	0.001
Head length	35	3.3	3.7	3.5±0.1	83	3.3	3.9	3.5±0.2	12	3.3	3.7	3.5±0.1	17	3.3	3.7	3.5±0.1	1.834	0.144
Head width	35	1.9	2.4	2.1±0.1^a	82	2.0	2.8	2.2±0.1^a	12	2.1	2.3	2.2±0.0^a	17	2.0	2.3	2.2±0.1^a	4.980	0.003
Ear length	35	2.1	2.6	2.4±0.1^a	83	1.4	2.8	2.4±0.2^a	12	2.1	2.7	2.3±0.2^a	17	1.7	1.9	1.8±0.1^b	64.726	0.001
Ear width	35	1.5	2.0	1.8±0.1^a	83	1.5	2.1	1.8±0.1^a	12	1.5	2.1	1.7±0.2^a	17	1.2	1.5	1.3±0.1^b	99.938	0.001
Tibia length	35	3.7	4.3	4.1±0.1^a	80	3.7	4.5	4.1±0.2^a	12	3.9	4.5	4.1±0.2^a	17	3.6	4.1	3.8±0.1^b	17.630	0.001
Hindfoot length	35	3.0	3.6	3.3±0.1^a	83	3.0	3.6	3.3±0.1^a	12	3.1	3.5	3.3±0.1^{ab}	17	3.0	3.3	3.2±0.1^b	6.247	0.001
Tail length/head length	35	3.9	5.0	4.5±0.3^a	82	3.4	5.8	4.1±0.4^b	12	3.6	4.6	4.1±0.3^b	17	3.5	4.2	3.8±0.2^c	20.106	0.001
Tail length/head width	35	6.1	8.5	7.3±0.5^a	81	4.8	7.5	6.6±0.5^b	12	5.8	7.5	6.6±0.5^b	17	5.5	6.7	6.1±0.3^c	25.552	0.001
PC 1	35			-3.78±0.76 ^a	79			0.08±1.03 ^a	12			-0.43±1.31 ^a	Not included				1.429	0.244
PC2	35			-0.32±0.81 ^a	79			0.11±1.09 ^a	12			0.21±0.74 ^a	Not included				2.543	0.083
PC3	35			-0.56±0.89^a	79			0.27±0.96^b	12			-0.15±0.89^{ab}	Not included				9.548	0.001

N = sample size; Min = minimum, Max = maximum, mean ± standard deviations. F-values indicate overall differences between characters according to ANOVA. Different superscripts (a, b, c) indicate differences between characters with $p < 0.05$ according to Scheffé’s post-hoc test.

Mg = *Microcebus griseorufus*; *Mm* = *M. murinus*

Table IV Comparison of body mass and head and body length of *Microcebus murinus* and *M. griseorufus* in allopatry and sympatry.

	Species	Sex	Allopatry				Sympatry				t-test	
			N	Min	Max	Mean±SD	N	Min	Max	Mean±SD	t	p
Body mass (g)	<i>M. griseorufus</i>	Female	19	42	72	53.2±7.8	3	48	55	51.0±3.6	0.474	0.640
		Male	11	47	60	53.6±3.6	3	42	51	47.0±4.6	2.687	0.020
	<i>M. murinus</i>	Female	27	47	90	64.9±11.3	12	47	86	62.9±12.6	0.485	0.631
		Male	28	47	70	57.5±6.5	12	47	64	54.3±5.0	1.538	0.132
Head and body length (cm)	<i>M. griseorufus</i>	Female	19	12	16	14.1±1.2	3	13	14.7	13.7±0.9	0.466	0.646
		Male	11	12.6	15	13.4±0.6	3	10.3	12.5	11.7±1.2	3.477	0.005
	<i>M. murinus</i>	Female	26	10.6	16	14.3±1.0	12	11.5	15.5	14.3±1.2	0.039	0.969
		Male	28	12.5	15	13.7±0.6	12	13	14.5	13.7±0.6	0.385	0.702

N = sample size; Min = minimum, Max = maximum, mean ± standard deviations. Significant differences are indicated in bold.

Microcebus murinus and *M. griseorufus* form hybrids with an intermediate morphological “Gestalt” (as summarized by a principal component analysis of the morphological measurements) in the dry vegetation formations inhabited by both species. In some of these vegetation formations, the two species occur in sympatry. In an area further west, at Tsimanampetsotsa National Park, *Microcebus griseorufus* is the only species present. There, it occupies all vegetation formations with higher densities in the more mesic formations, such as dry deciduous forest growing along the lake (Rakotondranary *et al.* 2010; Bohr *et al.* in press). The data from Tsimanampetsotsa illustrate that *Microcebus griseorufus* can do well in mesic vegetation formations and does better there than in the drier vegetation formations such as dry spiny forest or spiny bush. Thus, the absence of *Microcebus griseorufus* in the more mesic vegetation formations at Andohahela could be explained by competitive exclusion of *M. griseorufus* by *M. murinus* from the more mesic sites. *Microcebus murinus*, in turn, seems to be excluded from the humid forest by the presence of *M. rufus*. Similar to the situation of *Microcebus griseorufus* at Tsimanampetsotsa, *M. murinus* also does well in evergreen rain forests where *M. rufus* is not present. This situation is found in Mandena littoral forests (an evergreen humid forest with 1600 mm annual rain fall), where *Microcebus rufus* is absent (Lahann *et al.* 2006). The sequence of *Microcebus* species turnover along the gradient could therefore be due to a series of competitive exclusions: *M. griseorufus* seems best adapted to very dry vegetation formations, as conditions become more mesic, it is replaced by *M. murinus*, which in turn, is substituted by *M. rufus* at the most humid end of the gradient. Thus, distinct species seem to have an evolutionary advantage within their respective vegetation type. To date, the ecological and physiological data available for these species do not provide any explanation for the selective advantage of one species over the other in the different vegetation formations. In particular, recent studies have revealed very high intraspecific variation in morphology and physiology with respect to energy metabolism and reproduction. This makes it almost impossible to draw conclusions about evolutionary advantages of “species-specific” life history traits from studies in different sites (see reviews for *Microcebus rufus*: Atsalis 2007; for *M. murinus*: Lahann *et al.* 2006; for *M. griseorufus*: Génin 2008; Kobbe *et al.* 2011).

The scenario outlined above implies strong competition between the two sister species of *Microcebus*: *M. griseorufus* and *M. murinus*. However, this is not reflected in changes in body measurements between populations in sympatry and allopatry as predicted by Hutchinson’s rule. Among sympatric species pairs of *Microcebus* studied so far, only *M. murinus* and *M. berthae*

differ in body mass by a factor of about 2 and thus match “Hutchinson’s rule” that postulates that differences in body mass are important to allow the coexistence of congeneric species. This mechanism does not appear to be true for *Microcebus murinus* and *M. griseorufus*. Only minor changes were observed in body mass and head and body length in *Microcebus griseorufus*. *Microcebus griseorufus* were smaller in sympatry than in allopatry. The direction of these changes could be interpreted as character displacement, but the degree of change was far from the difference postulated to reduce competition to the extent that would allow coexistence (Hutchinson 1959; Ganeshaiah *et al.* 1999).

CONCLUSION

Andohahela provides an excellent opportunity to investigate the evolutionary phenomena that are thought to have led to the radiation of lemurs and other taxa. The ecotone at the interface of the evergreen humid and the dry spiny forest represents a very clear border between *Microcebus rufus* and *M. murinus* without any indication of hybridization. The lack of hybridization may also be a consequence of their long divergence some 5 – 12 million years ago. This clear species separation supports the “current climate hypothesis”. Situations where the much younger sister species *Microcebus murinus* and *M. griseorufus* come into contact are more complex. Where gallery and dry spiny forests form clear boundaries due to rivers with permanent water (such as in Ambatoabo or Tsimelahy), the two species are clearly separated and the incidence of hybrids is low. This indicates that processes associated with the “watershed hypotheses” can also produce species boundaries. At sites where vegetation formations are poorly defined and form a small scale mosaic of different vegetation types, hybrids are more common. Thus, the evolution of distinct taxa seems to require distinct vegetation formations, regardless whether ecotones are generated by climate zones or by edaphic phenomena. Where congeneric species occur in sympatry, competition seems to be avoided primarily by differences in food composition.

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Electronic Supplementary Material S1 Study sites in and around Andohahela National Park.

Site name	Transect name	Starting point			End point			Vegetation type
		Southern Latitude	Eastern Longitude	Altitude (m)	Southern Latitude	Eastern Longitude	Altitude (m)	
Hazofotsy	T01	S24°50'18.99''	E046°31'37.74''	88	S24°50'32.17''	E046°31'46.06''	96	Dry spiny forest
	T02	S24°49'47.57''	E046°32'46.07''	97	S24°49'38.94''	E046°33'00.20''	100	Dry spiny forest
	T03	S24°49'52.34''	E046°32'16.70''	84	S24°49'49.90''	E046°32'31.72''	87	Dry spiny forest
Ambatoabo	T04	S24°49'14.20''	E046°40'05.38''	154	S24°49'07.45''	E046°39'49.33''	142	Dry spiny forest
	T05	S24°49'10.08''	E046°40'07.66''	136	S24°49'05.96''	E046°40'23.16''	141	Gallery forest
	T06	S24°49'03.17''	E046°40'16.45''	150	S24°48'58.01''	E046°40'28.56''	150	Dry spiny forest
Ankoba	T07	S24°47'59.33''	E046°41'22.76''	209	S24°48'13.24''	E046°41'21.52''	240	Transitional forest
	T08	S24°47'45.07''	E046°41'05.96''	172	S24°47'51.58''	E046°40'51.95''	140	Gallery forest
	T09	S24°47'42.67''	E046°41'17.72''	191	S24°47'35.70''	E046°41'31.60''	193	Transitional forest
Mahamavo	T10	S24°43'30.38''	E046°43'27.19''	730	S24°43'45.78''	E046°43'26.71''	666	Rain forest
	T11	S24°44'10.76''	E046°43'19.83''	573	S24°43'59.07''	E046°43'28.49''	630	Rain forest
	T12	S24°44'12.46''	E046°43'23.24''	575	S24°44'27.51''	E046°43'24.29''	525	Rain forest
Mangatsiaka	T13	S24°58'1.44''	E046°33'15.54''	82	S24°58'12.24''	E046°33'20.52''	68	Gallery forest
	T14	S24°58'03.60''	E046°33'19.56''	80	S24°58'9.12''	E046°33'33.66''	67	Dry spiny forest
	T15	S24°58'00.79''	E046°33'15.44''	82	S24°57'50.49''	E046°33'28.08''	104	Dry spiny forest
	T16	S24°57'49.89''	E046°33'28.96''	105	S24°57'38.72''	E046°33'36.61''	140	Dry spiny forest
	T17	S24°58'06.25''	E046°33'45.50''	74	S24°58'06.46''	E046°34'01.71''	76	Dry spiny forest
	T18	S24°58'00.09''	E046°33'12.47''	71	S24°57'44.86''	E046°33'12.34''	88	Gallery forest
	T19	S24°57'43.85''	E046°33'12.09''	103	S24°57'29.32''	E046°33'11.07''	119	Dry spiny forest
	T20	S24°57'49.33''	E046°33'15.84''	104	S24°57'43.10''	E046°33'30.82''	106	Gallery & dry spiny forest
Tsimelahy	T21	S24°57'19.48''	E046°37'09.14''	123	S24°57'31.75''	E046°36'59.95''	115	Gallery forest
	T22	S24°56'52.67''	E046°37'16.57''	159	S24°57'04.02''	E046°37'05.31''	170	Gallery forest
	T23	S24°57'3.85''	E046°37'4.51''	147	S24°57'14.9''	E046°36'57.71''	158	Dry spiny forest
	T24	S24°57'17.85''	E046°36'54.52''	163	S24°57'22.08''	E046°36'38.14''	159	Dry spiny forest
	T25	S24°57'15.66''	E046°37'15.46''	128	S24°57'14.04''	E046°37'9.19''	164	Gallery & dry spiny forest
	T26	S24°57'20.65''	E046°36'48.31''	156	S24°57'22.94''	E046°37'02.23''	137	Gallery & dry spiny forest
Ebosika	T27	S24°56'32.53''	E046°39'47.59''	318	S24°56'37.39''	E046°39'47.05''	306	Transitional forest
	T28	S24°56'33.25''	E046°39'36.36''	290	S24°56'31.06''	E046°39'28.59''	261	Transitional forest
	T29	S24°56'38.7''	E046°40'17.28''	412	S24°56'27.63''	E046°40'26.03''	622	Rain forest
	T30	S24°56'40.80''	E046°40'16.28''	389	S24°56'43.74''	E046°40'32.34''	446	Rain forest

Chapter 1: Separation of *Microcebus* species along an environmental gradient

S2 Temperature and humidity at different sites and vegetations in the Andohahela National Park (hot season: data from December 2008 and January 2009; cold season: data from June and July 2008).

	Temperature (°C)						Humidity (%RH)					
	December - January			June - July			December - January			June - July		
	Rain forest	Gallery forest	Dry spiny forest	Rain forest	Gallery forest	Dry spiny forest	Rain forest	Gallery forest	Dry spiny forest	Rain forest	Gallery forest	Dry spiny forest
Mahamavo	N	744		732			744					
	Min	16.1		11.1			26.8					
	Max	38.1		24.6			100.0					
	Mean±SD	24.6±4.0		16.2±2.6			78.2±16.2					
Ebosika	N	744		732			744					
	Min	18.7		11.6			24.8					
	Max	45.1		23.2			100.0					
	Mean±SD	27.5±5.1		17.0±2.2			72.0±17.8					
Ankoba	N	744	744	732	732		744	744				
	Min	18.6	19.7	9.2	11.7		19.1	16.5				
	Max	45.1	42.6	26.7	29.7		100.0	100.0				
	Mean±SD	29.3±5.4	29.7±4.4	19.2±3.6	19.7±3.4		64±20.1	60.0±18.3				
Ambatoabo	N	744	744(*)	732	732		744					
	Min	20.2	20.1	8.6	8.6		30.8					
	Max	36.1	41.6	26.6	33.6		95.9					
	Mean±SD	27.4±3.4	29.4±4.6	19.2±3.4	20.8±4.9		70.2±14.6					
Tsimelahy	N	744	744	732	732		744	720		732	732	
	Min	19.2	20.2	9.6	11.6		18.4	29.7		21.2	30.7	
	Max	45.2	38.7	28.2	29.1		100.0	100.0		100.0	100.0	
	Mean±SD	29.3±5.6	28.6±4.2	18.5±3.8	19.6±3.5		65.9±21.3	69.2±17.5		77.2±14.5	76.0±14.0	
Mangatsiaka	N	744	744	732	570		744	744		732	570	
	Min	20.2	19.7	10.6	12.7		21.2	20.2		14.6	9.6	
	Max	41.1	43.2	29.2	32.7		100.0	100.0		100.0	100.0	
	Mean±SD	29.2±4.8	29.0±5.5	19.1±4.2	20.2±4.1		63.9±19.5	68.2±21.6		71.6±16.3	69.2±19.3	
Hazofotsy	N		744		732			660			732	
	Min		22.2		12.7			12.9			9.5	
	Max		48.1		38.2			100.0			100.0	
	Mean±SD		32.7±5.9		22.7±5.4			55.4±23.8			58.2±17.0	

N = number of recordings; Min = minimum, Max = maximum, mean ± standard deviations. (*) Data from December 2007 and January 2008

S3 Differences in body mass and morphological measurements between species and sex; F and p-values are based on ANOVA. Significant effects are marked in bold.

	Species		Sex		Species x Sex	
	F	p	F	p	F	p
Body mass	19.224	0.001	1.659	0.200	2.690	0.049
Head and body length	12.669	0.001	4.649	0.033	1.339	0.264
Tail length	26.363	0.001	0.214	0.644	1.236	0.299
Head length	2.051	0.110	1.350	0.247	0.006	0.999
Head width	5.812	0.001	1.294	0.257	0.505	0.679
Ear length	67.376	0.001	0.756	0.386	1.460	0.228
Ear width	104.435	0.001	4.095	0.045	0.973	0.407
Tibia length	17.853	0.001	0.044	0.834	0.555	0.646
Hindfoot length	6.472	0.001	0.846	0.359	0.958	0.415

S4 Factor loadings of principal components summarizing morphological traits of *Microcebus griseorufus*, *M. murinus* and hybrids between the two.

Variable	PC1	PC2	PC3
Head and body length	0.574	0.260	0.311
Tail length	0.556	0.086	-0.588
Head length	0.381	0.644	0.104
Head width	0.157	0.618	0.522
Ear length	0.589	-0.541	0.308
Ear width	0.450	-0.558	0.428
Tibia length	0.768	0.014	-0.067
Hindfoot length	0.680	0.031	-0.0436
% of variance	30.1%	18.5%	15.0%

Regional, seasonal and interspecific variation in ^{15}N and ^{13}C in sympatric mouse lemurs

Naturwissenschaften (submitted)

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Abstract

Madagascar provides some of the rare examples where two or more primate species of the same genus and with seemingly identical niche requirements occur in sympatry. If congeneric primate species co-occur in other parts of the world, they differ in size in a way that is consistent with Hutchinson's rule for coexisting species. In some areas of Madagascar, mouse lemurs do not follow this "rule" and thus seem to violate one of the principles of community ecology. In order to understand the mechanisms that allow coexistence of sympatric congeneric species we studied food composition of two identical sized mouse lemur species, *Microcebus griseorufus* and *M. murinus* with the help of stable isotope analyses ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$). During the rich season, when food seems abundant, the two species do not differ in their nitrogen isotope composition, indicating that the two species occupy the same trophic level. However, during the lean season, *Microcebus murinus* consumes less animal matter than *M. griseorufus*. Hybrids between the two species showed intermediate food composition. The results reflect subtle differences in foraging

that are difficult to quantify by observations or fecal analyses but that represent one possibility to allow coexistence of species.

Keywords: Stable isotopes, competition, trophic level, *Microcebus*, Madagascar

Introduction

According to classical hypothesis on the composition of animal and plant communities, species can not coexist if they occupy the same ecological niche (Gause 1934). Competition over similar resources can be mediated and thus coexistence can be facilitated by size differences of the consumer species by a factor of 1.4 in size or by a factor of 2 in body mass, the so-called Hutchinson's rule (Hutchinson 1959; Simberloff and Dayan 1991). Alternatively, interspecific competition over similar resources results in diverging adaptations in morphology, physiology and behaviour, resulting in speciation (e.g., Darwin finches [Grant and Grant 2006, 2010], bats [Kalko 1998; Siemers and Swift 2006]). These classical mechanisms may not be operational in systems where species are not limited by resources, such as in ephemeral tadpole communities (Strauss *et al.* 2010).

Among long-lived species, such as primates, congeneric species rarely occur in sympatry as they are closely related phylogenetically and therefore are likely to share many adaptations (Houle 1997). If congeneric primate species co-occur, they differ in size or niche dimensions in ways that are consistent with Hutchinson's rule for coexisting species or differences in ecology (Fleagle 1998; Schreier *et al.* 2009). While most of Madagascar's lemur communities match this pattern (Ganzhorn 1989; Ganzhorn *et al.* 1999), mouse lemurs (*Microcebus* spp.) represent some remarkable exceptions that question the general validity of the present conception on the mechanisms that structure animal communities. In several areas of Madagascar two species of the genus *Microcebus* coexist. Examples are: *M. murinus* and *M. berthae* that differ in size, habitat utilization and food composition (Schwab and Ganzhorn 2004; Dammhahn and Kappeler 2008, 2010); the coexisting *M. murinus* and *M. ravelobensis* show no size differences but are separated by habitat utilization and food composition (Rakotondravony and Radespiel 2009; Thorén *et al.* 2011). These species pairs are phylogenetically distinct as *M. murinus* is a member of the western, dry ecosystem clade and *M. berthae* and *M. ravelobensis* are rooted in the eastern clade (Yoder and Yang 2004). In contrast to the previous examples, *M. murinus* and *M. griseorufus* are sibling

species that are sympatric in parts of their range (Yoder and Yang 2004). Though *M. griseorufus* occur primarily in the drier parts of the habitat while *M. murinus* occupy the wetter sites, such as gallery forests (Yoder *et al.* 2002; Génin 2008; Rakotondranary *et al.* 2011), the two forms mix in some areas and form hybrids (Gligor *et al.* 2009). Nevertheless they are genetically distinct outside the small zones of hybridization (Hapke *et al.* submitted), thus indicating subtle differences in their adaptations to cope with their joint environment.

Stable carbon and nitrogen isotope analysis of animal tissues is as powerful tool to investigate dietary patterns and trophic relationships within ecosystems (DeNiro 1987, Peterson and Fry 1987, Struck *et al.* 2002; Herrera *et al.* 2003) with the possibility to disentangle very fine differences between species (Siemers *et al.* 2011). This technique is based on the fact that the isotopic composition of the nitrogen and carbon in an animal reflects the nitrogen isotopic composition of its diet (DeNiro and Epstein 1978, 1981; Eggers and Jones 2000). Studies of animals fed diets of known isotopic composition have demonstrated that the stable carbon and nitrogen isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of an animal's tissues increase with increasing trophic level (DeNiro and Epstein 1978, 1981). Enrichments are 1–2‰ for $\delta^{13}\text{C}$ -values (DeNiro and Epstein 1978) and 2–5‰ for $\delta^{15}\text{N}$ -values (DeNiro and Epstein 1981). Thus, this technique allows the analysis of complex trophic relationships (Eggers and Jones, 2000; Post *et al.*, 2000) and analysis of niche separation of animals (e.g., Siemers *et al.* 2011), including primates (e.g., Schoeninger *et al.* 1998; Loudon *et al.* 2007; Crowley *et al.* 2011) and on two sympatric mouse lemurs in Madagascar in particular (Dammhahn and Kappeler 2010). In a nation-wide analysis, Crowley *et al.* (in press) demonstrated that the isotope signature of C_3 plants is highly correlated with the isotope signature in hair of *Microcebus*, explaining some 99.7% of the variation between sites. This spatial variation is supplemented by seasonal variation of the isotope composition in animal tissue (Dammhahn and Kappeler 2010).

Our aim is to investigate possible dietary differences as a mechanism either maintaining species identities or towards the evolution of two mouse lemur species (*M. griseorufus* and *M. murinus*) with the help of stable carbon and nitrogen isotope analyses. The following questions were explored in this study:

1. Do the isotope signatures of a given species vary in relation to environmental conditions?
2. In allopatry, do the two species of *Microcebus* show seasonal changes in isotope signatures?

3. In sympatry, do the two species of *Microcebus* show different isotope signatures indicating dietary niche separation?
4. Do fecal analyses reflect possible dietary differences between species?

Materials and methods

Study Sites

The study was carried out in the evergreen littoral rain forest of Mandena and in the forests of National Park of Andohahela (Parcel n°1, Parcel n°2 and between Parcel n°1 and n°2) in the south of Madagascar (Rakotondranary *et al.* 2011). Annual rainfall of the littoral rain forest of Mandena is 1540 mm per year (Vincelette *et al.* 2007). Based on the vegetation classification of Moat and Smith (2007), three types of vegetations were selected in Andohahela namely the rain forest, gallery forest, and dry spiny forest. The National Park of Andohahela is characterized by a steep rainfall gradient from 400 mm in the west to 2400 mm per year in the east (Barthlott *et al.* 1996; Goodman 1999). Temperature data and air humidity measured in the dry spiny forest, the gallery and the rain forest indicate a continuous decline of the ambient temperature and a continuous increase of the humidity from the spiny forest to the rain forest. Gallery forests are within the domain of dry spiny forest but have higher air humidity and lower ambient temperature than the surrounding dry spiny forest due to groundwater availability and lush vegetation (Rakotondranary *et al.* 2011). Annual rainfall has not been measured at the sites of Andohahela, but the measurements of air humidity in the rainforest of Andohahela are similar to measurement close to Mandena (Vincelette *et al.* 2007; Rakotondranary *et al.* 2011). Since the rain forest site at Andohahela was at an altitude of 570 – 730 m a.s.l., ambient temperature was lower than at any of the other sites. Thus the vegetation formations are characterized by increasing rainfall from the dry spiny forest – gallery forest – rain forest (Andohahela) and littoral rain forest (Mandena) and in terms of increasing ambient temperature from rain forest – littoral forest – gallery forest – dry spiny forest.

Sampling

From September 2006 to June 2009 we captured *Microcebus* spp. at the different sites during the end of the dry season (September, October, November) and after the birth season at the end of the wet season (April, May, June). Trapping was performed with Sherman traps (7.7 × 7.7 × 23 cm)

placed on a tree. Traps were baited with bananas for four successive nights per transect. In Mandena we used standard trapping grids in fragment M16 (Ramanamanjato and Ganzhorn 2001). In the field, animals were classified based on morphological criteria (Rakotondranary *et al.* 2011). Identifications were verified on the bases of mitochondrial DNA and microsatellites (Hapke *et al.* submitted). In Andohahela, we established 19 transect composed of 20 trapping points, spaced at 25 m intervals. From every *Microcebus* spp captured, we sampled hair and feces. Hair samples were stored without any preservative. Fecal samples were collected from the traps at the first night of capture and stored in 70% ethanol or without any preservative.

Stable isotope analysis

Hair samples collected in the months of September, October and November are considered to represent the food composition of the preceding dry season. Samples collected in April and May are considered to represent the food composition of the wet season. Samples were oven dried to constant weight at 60° C. Stable nitrogen and carbon isotope analysis and concentration measurements of hair samples from Andohahela were performed with a THERMO/Finnigan MAT V isotope ratio mass spectrometer, coupled to a THERMO Flash EA 1112 elemental analyzer via a THERMO/Finnigan Conflo III- interface in the stable isotope laboratory of the Museum für Naturkunde, Berlin. Isotopic values are expressed in the δ -notation relative to primary standards VPDB (Vienna Pee Dee belemnite) for $\delta^{13}\text{C}$ and atmospheric nitrogen (air) for $\delta^{15}\text{N}$ (Mariotti 1983) according to:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{std}}) - 1] \cdot 1000 (\text{‰}),$$

with R_{sample} being the ratio between $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and R_{std} the ratio between $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the standards.

Standard deviation for repeated measurements of lab standard material (peptone) is generally below 0.15 (‰) for nitrogen and carbon. Standard deviations of concentration measurements of replicates of lab standards are < 3% of the concentration analyzed.

Stable carbon and nitrogen isotope signatures of the samples from Mandena were analyzed at the Institute of Soil Science of the University of Hamburg using a Flash 2000 elemental analyzer coupled via a Conflo IV to a Delta V Plus (Thermo Scientific, Germany) isotope ratio mass spectrometer. The standard deviation of replicate measurements was less than $\pm 0.2\text{‰}$. $\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$ -values were calculated using the external standards USGS40 ($\delta^{13}\text{C}$ -26.4‰ VPDB, $\delta^{15}\text{N}$ - 4.5‰ air), IAEA-C6 ($\delta^{13}\text{C}$ -10.8‰ VPDB), and IAEA-N1 ($\delta^{15}\text{N}$ + 0.4 air).

Results from samples analyzed by both labs differed by 0.37 ± 0.17 ‰ and -0.29 ± 0.12 for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. Results for $\delta^{15}\text{N}$ were higher and results for $\delta^{13}\text{C}$ were lower in Berlin than in Hamburg. These differences were small, consistent and significant (paired t-tests: $t > 4.4$; $P < 0.05$; $n = 4$). Accounting for these differences does not change the results. Therefore we used the original data as provided by the laboratories for the analyses.

Fecal analysis

Fecal samples were analyzed for fruit and insect remains with a microscope. The presence and percentage of different food categories were estimated.

Statistical Analysis

Parametric analysis of variance (ANOVA) and Scheffe's post-hoc were used to analyze isotope signatures among *Microcebus* populations in relation to environmental conditions. Differences between *Microcebus* species were analyzed with t-tests using SPSS 13.0. *P*-values of multiple pairwise comparisons were Bonferroni corrected.

Results

No significant differences, neither in $\delta^{13}\text{C}$ nor in $\delta^{15}\text{N}$ values, were found between the sexes. Therefore we did not consider the sexes effects separately in the analyses.

1. Stable nitrogen and carbon isotope of *M. murinus* in relation to environmental conditions

Only *M. murinus* occurs in all vegetation types of the region, such as the littoral rain forest of Mandena; and the rain, gallery and dry spiny forest of Andohahela, we compared $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of different populations of this species from different vegetation types. For this comparison we used only sites where *M. murinus* was the only *Microcebus* species present to avoid confounding effects due to possible interspecific competition over food resources. The comparison is based on 22 individuals for the wet season and 25 individuals for the dry season (Fig. 1). $\delta^{15}\text{N}$ values differed between populations in the wet season (ANOVA: $F = 6.67$, $P = 0.018$)

and in the dry season (ANOVA: $F=23.53$, $P<0.001$). $\delta^{13}\text{C}$ values were not different (Fig. 1). $\delta^{15}\text{N}$ values were highest in dry spiny forest and lowest in the rain forest.

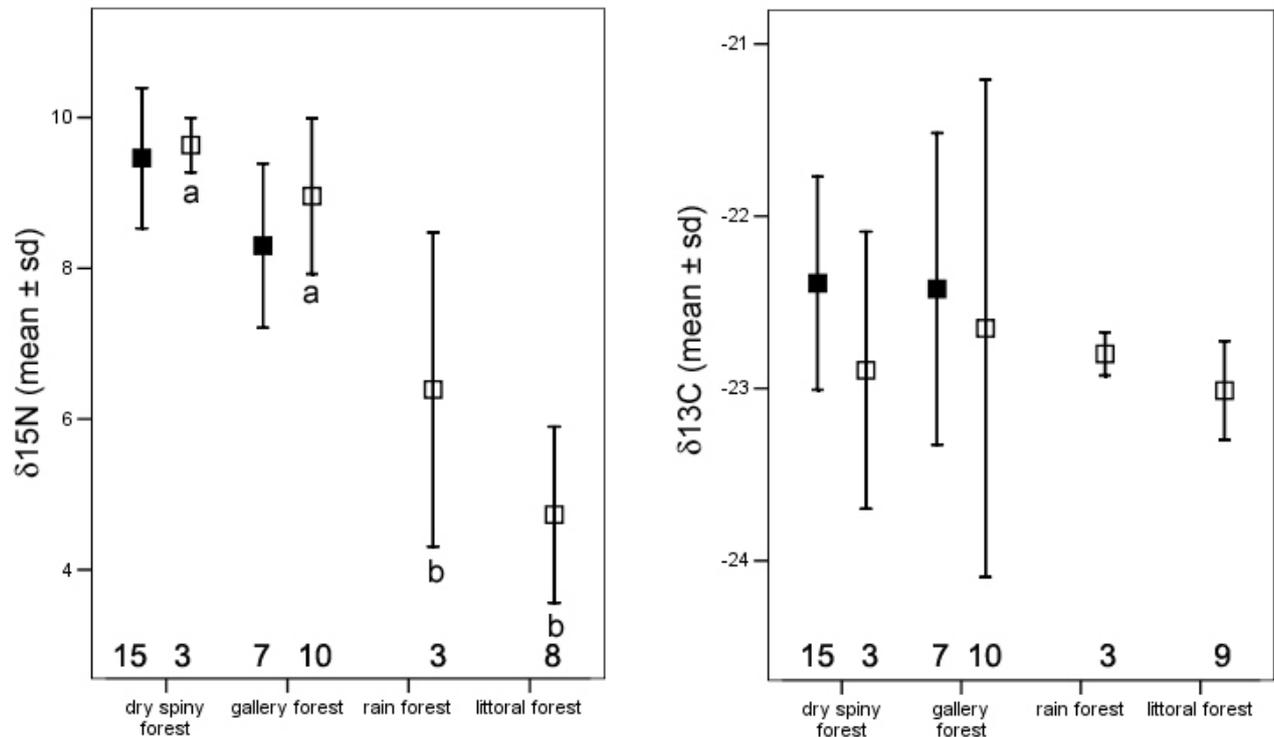


Fig. 1 Mean \pm standard deviation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between populations of *M. murinus* living in allopatry in different habitats (■ wet season; □ dry season). The letters below the error bars indicate differences between sites during the dry season with $P<0.05$ according to Scheffé's post-hoc test. Numbers indicate sample size.

2. Seasonal variation and interspecific differences in isotope signatures among mouse lemur in allopatry

The comparisons of the isotope signatures between and within species in allopatry in different season are based on 43 individuals in the dry spiny forest. In allopatry, isotope signatures vary between species (Fig. 2, 3). Here, *M. griseorufus* had higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than *M. murinus* during wet season (t -tests, $\delta^{15}\text{N}$: $t=6.72$, $P<0.001$; $\delta^{13}\text{C}$: $t=5.12$, $P<0.001$) and during the dry season (t -tests, $\delta^{15}\text{N}$: $t=8.42$, $P<0.001$; $\delta^{13}\text{C}$: $t=4.01$, $P=0.001$). Isotope signatures remain constant between seasons within species.

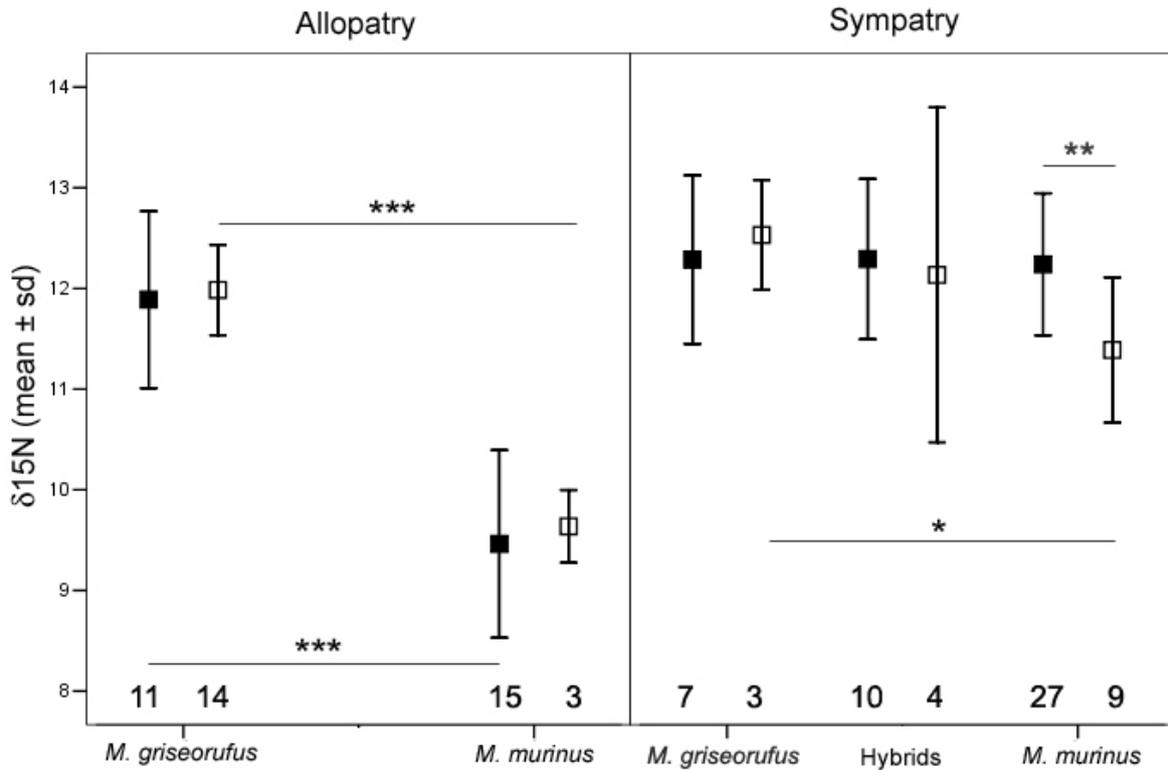


Fig. 2 Within and between species differences in stable nitrogen isotope ($\delta^{15}\text{N}$) values (■ wet season; □ dry season). Differences are indicated by lines and * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Numbers indicate sample size.

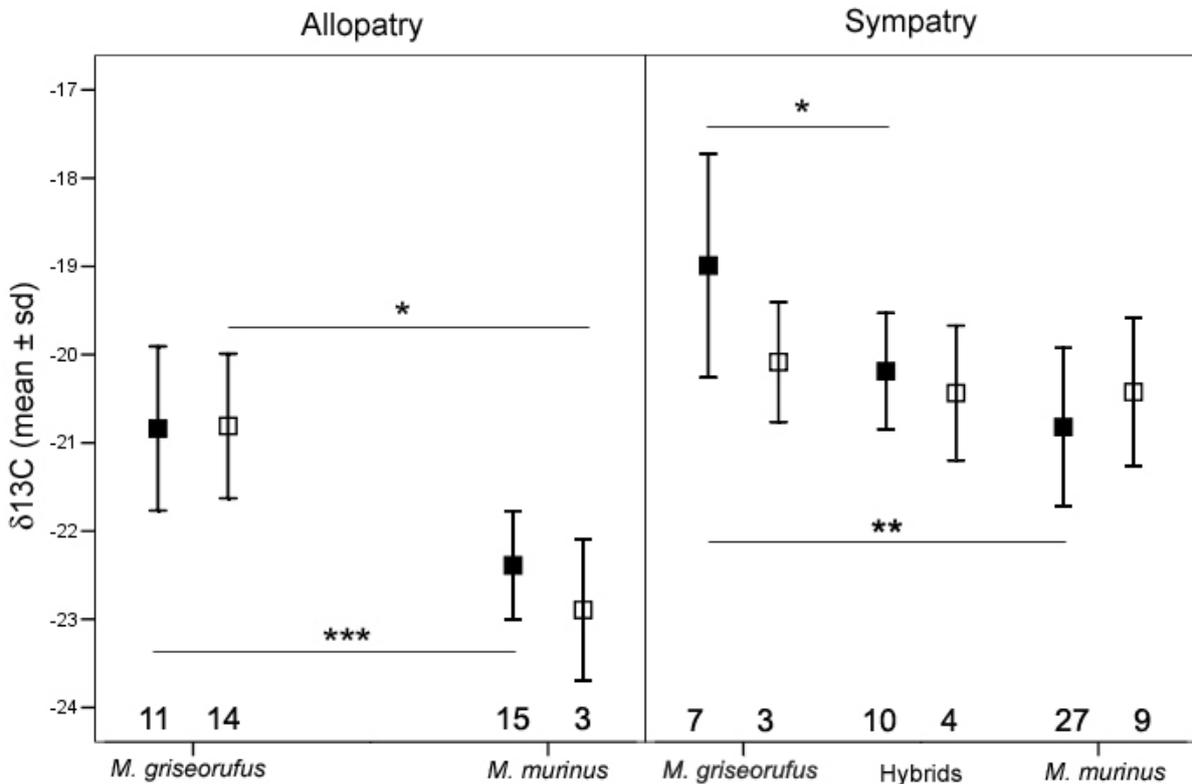


Fig. 3 Within and between species differences in stable carbon isotope ($\delta^{13}\text{C}$) value (■ wet season; □ dry season). Differences are indicated by lines and * $P < 0.05$, ** $P < 0.01$. Numbers indicate sample size.

3. Seasonal variation and interspecific differences in isotope signatures among mouse lemur in sympatry

The comparisons of the isotope signatures between and within species in sympatry in different season are based on 60 individuals in the dry spiny forest. In sympatry, $\delta^{15}\text{N}$ did not differ between species during the wet season (Fig. 2, 3). However, during the dry season, the $\delta^{15}\text{N}$ value of *M. griseorufus* was significantly higher than the value of *M. murinus* (*t*-test: $t=2.48$, $P=0.032$). The $\delta^{15}\text{N}$ value of *M. murinus* was lower during the dry than during the wet season (*t*-test: $t=3.11$, $P=0.004$).

In sympatry, $\delta^{13}\text{C}$ did not differ between species during the dry season. During the wet season *M. griseorufus* had higher $\delta^{13}\text{C}$ values than *M. murinus* (*t*-test: $t=4.42$, $P<0.002$) and hybrids (*t*-test: $t=2.56$, $P=0.04$; *P*-values corrected according to Bonferroni). Hybrids had similar $\delta^{13}\text{C}$ values as *M. murinus* (*t*-test: $t=2.03$, $P>0.05$ after Bonferroni correction). No species showed a significant difference between seasons with respect to $\delta^{13}\text{C}$ values.

Fecal analysis

Fecal analyses were based on 47 individuals (Table 1). Insects debris were present in feces of all individuals (*M. murinus*, *M. griseorufus* and hybrids). Seeds were only found in feces of *M. murinus*. Due to small sample sizes, we did not conduct statistical tests.

Table 1 Fecal analyses of *Microcebus* spp. at Andohahela

Species	Vegetation	Life-style	Season	N	Seed presence	Insecte presence	Proportion of insect debris	
							$\leq 50\%$	$> 50\%$
<i>M. griseorufus</i>	Dry spiny forest	Allopatry	Dry season	10	0	7	2	5
	Dry spiny forest	Sympatry	Dry season	2	0	2	0	2
<i>M. murinus</i>	Gallery forest	Allopatry	Wet season	1	0	1	0	1
	Gallery forest	Allopatry	Dry season	7	0	7	3	4
	Dry spiny forest	Allopatry	Dry season	7	0	4	2	2
	Dry spiny forest	Sympatry	Wet season	12	6	12	6	6
	Dry spiny forest	Sympatry	Dry season	4	1	3	1	2
Hybrids	Dry spiny forest	Sympatry	Wet season	2	1	1	1	0
	Dry spiny forest	Sympatry	Dry season	2	0	2	2	0

Discussion

Environmental effects

The $\delta^{15}\text{N}$ values measured in different populations of *M. murinus* showed systematic differences in relation to the ambient conditions in different vegetation types. $\delta^{15}\text{N}$ increased with decreasing rainfall and increasing temperature. Since the analyses were based only on *M. murinus* in allopatry, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were not influenced by possible competition between species and subsequent differences in diet composition. A similar relationship between ambient conditions and isotope compositions has been described in a nationwide comparison of $\delta^{15}\text{N}$ values in hair of different species of *Microcebus* and C_3 plants growing at these sites (Crowley *et al.* in press) and also elsewhere (Austin and Vitousek 1998). High $\delta^{15}\text{N}$ values in animals have been attributed to nutrient and water stress (Cormie and Schwarcz 1996). The $\delta^{13}\text{C}$ values were also correlated with annual rainfall, but differences between sites were small. The principal difference of the $\delta^{13}\text{C}$ value is based on photosynthetic pathways. C_4 and CAM plants are isotopically distinct from C_3 plants, with C_4 and CAM plants having higher $\delta^{13}\text{C}$ values than C_3 plants (Smith and Epstein 1971, Troughton and Card 1975). At Andohahela, the dry spiny forest is characterized by high abundances of Didiereaceae, Burseraceae and Euphorbiaceae, whereas Rubiaceae, Anacardiaceae and Myrtaceae are abundant in gallery and humid forest (Andriaharimalala *et al.* in press). CAM is the major photosynthetic pathway operating in the Didiereaceae and Euphorbiaceae of southern Madagascar (Winter 1979). Thus, while $\delta^{13}\text{C}$ values require in depth studies on the source of variation, due to soil conditions and different photosynthetic pathways, $\delta^{15}\text{N}$ values seem to provide a useful tool to reconstruct ambient habitat conditions (Crowley *et al.* in press).

Seasonal variation and interspecific dietary differences

Stable isotope ratios have been used successfully to determine the dietary bases for a large range of animals at various trophic levels (e.g., Post 2002; Cherel *et al.* 2007; Siemers *et al.* 2011), including primates (Schoeninger *et al.* 1998; Loudon *et al.* 2007; Dammhahn and Kappeler 2010; Crowley *et al.* in press). In general, $\delta^{15}\text{N}$ values increase by about 3‰ from one trophic level to the next, and $\delta^{13}\text{C}$ values increase by 1 - 2‰ (DeNiro and Epstein 1981). Thus, $\delta^{15}\text{N}$ values are fairly reliable indicators of trophic levels, while different $\delta^{13}\text{C}$ values can have a number of different sources apart from the trophic position of an animal in the food web. Furthermore, taxonomic and thus metabolic vegetation differences are passed on in the food chain and are

likely to be reflected in the isotope signatures of *M. murinus* and *M. griseorufus*. Since *M. griseorufus* is more likely to inhabit the dryer vegetation formations, the principal plants foods of *M. griseorufus* and their prey are C_4 and CAM plants, whereas *M. murinus* lives in more mesic vegetation systems with abundant C_3 plants (Rakotondranary *et al.* 2011). In allopatry, the question of competition between two species is excluded. Therefore, the composition of their diet should be based on preferences and the availability of food items.

Due to possible vegetation effects on the isotope signature, we analyzed animals living in different types of vegetation separately. In allopatry, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values do not change within species between seasons. *Microcebus griseorufus* show $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values that are 3‰ and 2‰ higher than in *M. murinus*. This would indicate that *M. griseorufus* occupies a higher trophic level (i.e. feeding more on insects) than *M. murinus*. However, the difference between species measured in different sites could also be due to geographical differences, though on a very small scale of a few hundred meters.

In sympatry, the two species do not differ in their $\delta^{15}\text{N}$ values during the wet season. But *M. murinus* lowered their $\delta^{15}\text{N}$ values during the dry season. At this time of the year, they also had lower $\delta^{15}\text{N}$ values than *M. griseorufus*. Though the interpretations are hampered by low number of samples, $\delta^{15}\text{N}$ values of hybrids show very large $\delta^{15}\text{N}$ -variation but at an intermediate level between both species, indicating much high dietary flexibility of the hybrids compared to their parent species. The $\delta^{13}\text{C}$ values did not differ between species and hybrids during the dry season but were significantly higher in *M. griseorufus* than in either one of the other taxa during the wet season. These phenomena indicate that species separation is achieved through different degrees of insectivory during the dry season even though this is phenomenon is not reflected in the analyses of fecal samples. The higher $\delta^{13}\text{C}$ values of *M. griseorufus* during the wet season can not be attributed to feeding at a higher trophic level than either *M. murinus* or their hybrids because otherwise the $\delta^{15}\text{N}$ values should also be elevated. Since C_4 and CAM plants have much higher $\delta^{13}\text{C}$ values than C_3 plants (see above), the relatively high $\delta^{13}\text{C}$ values of *M. griseorufus* is either likely due to their feeding on plants with different photosynthetic pathways or a higher trophic level.

The present analysis illustrates the power of stable isotope analyses for the study of trophic structures in animal communities. At the same time, it demonstrates isotopic differences between

species on a very small geographical scale that have nothing to do with different trophic levels but are simply the consequence of changing environmental conditions over sharp ecotones.

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Habitat separation of sympatric *Microcebus* spp. in the dry spiny forest of south-eastern Madagascar

Folia Primatologica (submitted)

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Abstract

Different habitat utilization allows species to coexist. Since habitat structures do not represent resources that are consumed, it remains unclear whether or not the structures measured in primate ecology are relevant *per se*, whether they represent proxies for other resources that are limited, or whether results on habitat separation are spurious and a matter of chance. We studied two species of *Microcebus*, *M. murinus* and *M. griseorufus* along a continuous vegetation gradient where populations of the two species occur in sympatry or in allopatry. The two species are considered generalists without any specific adaptation for certain vegetation structures. In allopatry, neither species showed any sign of microhabitat selectivity. In sympatry, they differed significantly and discriminated against certain habitat structures: *M. murinus* was found in microhabitats with larger trees than average while *M. griseorufus* utilized microhabitats with smaller trees than average. We interpret this difference as a consequence (or prerequisite) for different energy saving strategies, such as torpor and hibernation.

Key Words: Competition, Habitat selection, Community ecology, Speciation, Primates, Lemurs

Introduction

Understanding the mechanisms that lead to distinct species distributions and allow ecologically similar species to coexist are fundamental issues in community ecology. According to the classical ideas, competition over limited resources is avoided through interspecific differences in habitat utilization, diet, or activity patterns (Schoener 1974). In addition, species can use the same type of resource if they differ in body mass by a factor of 2 or in linear measurements by a factor of 1.4 (Hutchinson 1959). These mechanisms seem to operate in and to structure most primate communities (Schreier et al. 2009). However, some sympatric species of mouse lemurs (*Microcebus* spp.) from Madagascar are of special interest as they seem to violate these assumptions at least in some communities where similar sized species coexist without obvious niche separation. In western Madagascar *Microcebus murinus* is a very widespread species, ranging from littoral humid forest in the south-east to the northwest. Its range overlaps with *Microcebus ravelobensis* in the northwest, *Microcebus berthae* in a small area of the Menabe, and *Microcebus griseorufus* in the south (Mittermeier et al. 2008).

These congeneric mouse lemur species differed in habitat utilization (Dammhahn and Kappeler 2008; Rendigs et al. 2003; Radespiel et al. 2006; Schwab and Ganzhorn 2004; Génin 2008; Rakotondranary et al. 2011), food composition (Dammhahn and Kappeler 2008, 2010; Rakotondravony and Radespiel 2009; Thorén et al. 2011; Rakotondranary et al., submitted), and activity patterns (Thorén et al. 2011). Different habitat utilization might come about due to different competitive potential of two sympatric species, such as in Ampijoroa where *M. murinus* outcompete *M. ravelobensis* (Thorén et al. 2010).

While these phenomena match the predictions of classical community ecology, the causal relationships between the distribution of the species and these environmental conditions remain enigmatic. For example, *M. murinus* is found in habitats ranging from evergreen humid forest to dry spiny forest with significant changes in life history traits (Lahann et al. 2006), and with populations being structured and patchily distributed within a seemingly uniform type of forest (Fredsted et al. 2004). These observations indicate that interspecific differences in habitat utilization are the consequence of yet unidentified limiting factors rather than a niche dimension that contributes to species separation *per se*.

We used *Microcebus murinus*, *M. griseorufus*, and their hybrids in the dry spiny forest at Andohahela National Park to investigate the role of habitat structure for the separation of sympatric congeneric mouse lemur. Along the vegetation gradient of Andohahela National Park *Microcebus murinus* and *M. griseorufus* show clear associations with specific habitat types; *Microcebus griseorufus* occurs in the dryer parts of the habitats while *M. murinus* is found in the more mesic sites (Yoder et al. 2002; Génin 2008; Rakotondranary et al. 2011). The two species hybridize at the ecotone between dry spiny and gallery forests (Gligor et al. 2009; Rakotondranary et al. 2011, Hapke et al. submitted). These seemingly clear habitat associations of the two species indicate that habitat structure could be a niche dimension for species separation.

In the paper, we address the following questions:

- 1) What are the characteristics of available habitats at sites where the species live in allopatry and at sites where species live sympatrically?
- 2) Are there differences in microhabitat structures used by the species and their hybrids in sympatry?
- 3) Are there indications for habitat selectivity by different *Microcebus* spp.?

Methods

Study sites

The study was conducted from September 2006 to June 2009 in Parcel n°2 and between Parcel n°1 and n°2 of Andohahela National Park (Rakotondranary et al. 2011). The vegetation of the study region is classified as dry spiny forest (Moat and Smith 2007) and characterized by a steep rainfall gradient from 400 mm in the west to 2400 mm per year in the east with pronounced vegetation differences on a the scale of a few kilometres (Goodman 1999; Rakotondranary et al. 2011).

Species identification

Microcebus murinus and *M. griseorufus* are sister species (Yoder and Zang 2004) that hybridize (Gligor et al. 2009). In the field, animals were classified based on morphological criteria (Rakotondranary et al. 2011). Identifications were verified on the bases of mitochondrial DNA

and microsatellites (Hapke et al. submitted). Capture and genetic verification of species identification was necessary to develop morphological criteria that will guarantee correct assignment of morphotypes to species and to identify hybrids.

Microhabitat utilisation

We captured mouse lemur at the different sites during the end of the dry season (September, October, November) and after the birth season at the end of the wet season (April, May, June). We selected one site where *Microcebus griseorufus* is the only *Microcebus* species (Hazofotsy), two sites where *Microcebus murinus* live in allopatry (Ambatoabo and Ankoba) and one site where *Microcebus griseorufus* and *M. murinus* occur in sympatry (Mangatsiaka) (Rakotondranary et al. 2011). Seven transects composed of 20 trapping points, spaced at 25 m intervals were established. Details on the transects and the vegetation are described by Rakotondranary et al. (2011) and Andriaharimalala et al. (in press). Since low capture rates in Ambatoabo and Ankoba prohibited statistical analyses, and since the vegetation of these sites was rather similar, these two sites were combined for the present analysis. At Mangatsiaka where mouse lemur species live in sympatry, we added a rectangular trapping grid with 200 trapping stations (10×20 stations spaced at 25-m intervals) to improve the spatial resolution. The coordinates of the south-western corner of the trapping grid were 24°57'52.27"S, 46°33'17.25"E. Trapping was performed with Sherman traps (7.7 × 7.7 × 23 cm) placed on a tree. Traps were baited with bananas for four successive nights. None of the measures used to characterise the vegetation structure of microhabitats where animals were caught differed between the seasons. Therefore we did not consider the seasons separately in subsequent analyses.

Selectivity for specific habitat structures was calculated as the value of microhabitats used by *Microcebus* spp. divided by the median of the variable in the representative sample of microhabitat descriptions for the grid and each transect. A value of 1 indicates no selectivity. The method follows that used by Andrianasolo et al. (2006) except that we used the median instead of the mean because our measurements of the representative samples deviated from normality.

Vegetation descriptions

The point-centred quarter method was used for microhabitat descriptions at each trapping point. For this, each sample point represents the centre of four compass directions that divide the sampling plot into four quarters. In each quarter, the distance from the centre of the nearest plant to the sampling point is measured ($d_1 - d_4$) (Ganzhorn et al. 2011). We measured two size classes of trees: trees ≥ 10 cm DBH (diameter at breast height) and trees of 5 - 9.9 cm DBH. Thus, four trees of each size class were recorded per sample point. Density of trees per hectare can be calculated as: Density (individuals / ha) = $10000 / d^2$ where d equals the mean distance of the four trees to the centre of the sampling point.

Statistical Analyses

Most vegetation measurements deviated significantly from normality in the representative samples. Therefore we used non-parametric Kruskal-Wallis Analysis of Variance and Mann-Whitney-U Tests for statistical comparisons of the representative samples. Significance levels for multiple comparisons were Bonferroni corrected. In contrast, all but one variable of the microhabitats used by *Microcebus* did not deviate from normality. Therefore we applied parametric analysis of variance (ANOVA) and Scheffe's post-hoc to compare microhabitats used by *Microcebus* spp. Each trapping station entered the analysis only once even when more than one animal had been caught at this station. We carried out the statistical tests using SPSS 13.0 for Windows.

Results

Vegetation structure of representative samples

All measures of vegetation structures differed significantly between sites (Table 1). Except for the DBH of large trees (≥ 10 cm DBH), the forest of Hazofotsy differs in all other vegetation characteristics from the forests of Mangatsiaka and Ambatoabo-Ankoba.

Table 1. Characteristics of representative samples of microhabitats of the different study sites. Values are medians and quartiles. N = number of microhabitat descriptions; χ^2 -values indicate overall differences between sites according to Kruskal-Wallis Analysis of Variance; *** p < 0.001. Different superscripts indicate significant differences between sites with p < 0.05 according to pairwise Mann-Whitney-U-Tests after Bonferroni correction.

	Hazofotsy	Mangatsiaka	Ambatoabo & Ankoba	χ^2
N	20	260	60	
Distance of large trees ≥ 10 cm DBH	5.5 ^a 2.7 / 4.0	3.3 ^b 2.6 / 4.2	3.1 ^b 2.4 / 3.9	23.14***
DBH of large trees ≥ 10 cm DBH	16.8 ^a 15.1 / 20.6	14.5 ^b 13.0 / 16.4	16.7 ^a 14.3 / 21.2	26.15***
Distance of small trees 5-9.9 cm DBH	5.7 ^a 4.4 / 7.0	2.6 ^b 2.2 / 3.3	2.5 ^b 2.0 / 2.9	45.73***
DBH of small trees 5-9.9 cm DBH	8.2 ^a 7.7 / 8.7	6.8 ^b 6.5 / 7.5	7.1 ^b 6.5 / 7.7	42.22***
Density (ind./ha) of large trees ≥ 10 cm DBH	343 ^a 208 / 652	925 ^b 567 / 1451	1032 ^b 643 / 1692	23.14***
Density (ind./ha) of small trees 5-9.9 cm DBH	313 ^a 203 / 515	1479 ^b 932 / 2114	1666 ^b 1169 / 2485	45.73***
Density (ind./ha) of all trees ≥ 5 cm DBH	655 ^a 444 / 1327	2646 ^b 1713 / 3868	2915 ^b 2125 / 3929	41.40***

Vegetation characteristic and selectivity of microhabitats by *Microcebus* spp.

Table 2 shows the vegetation characteristic of microhabitats used by *Microcebus* spp. Transformation of the raw data into selectivity indices revealed that in allopatry neither *Microcebus griseorufus* nor *M. murinus* used microhabitats that differed in their structures from the microhabitats that were available at Hazofotsy and Ambatoabo & Ankoba, respectively. But both species showed significant deviations from the representative samples where they occur in sympatry at Mangatsiaka. At Mangatsiaka, *M. griseorufus* were caught in microhabitats with higher densities especially of small trees and large trees had lower diameters than the trees from the representative sample (Table 3). *Microcebus murinus* was found in microhabitats where trees had larger DBH than in the representative samples. Hybrids did not show any selectivity in relation to habitat structures.

Table 2. Occurrence of *Microcebus* spp. and vegetation characteristic of microhabitats used by *Microcebus* spp. Values are means and standard deviations. N = number of microhabitats where *Microcebus* were caught. Figures in bold indicate statistically significant differences between taxa in sympatry. Different superscripts indicate differences between taxa with $p < 0.05$ according to Scheffe's post-hoc test. Statistical tests were only performed for the differences between taxa in sympatry.

	Hazofotsy		Mangatsiaka		Ambatoabo & Ankoba
	(allopatric) <i>M. griseorufus</i>	<i>M. griseorufus</i>	(sympatric) <i>M. murinus</i>	Hybrids	(allopatric) <i>M. murinus</i>
N	9	21	65	28	6
Distance of large trees ≥ 10 cm DBH	5.7 ± 1.9	3.4 ± 1.2	3.8 ± 1.7	3.5 ± 1.3	3.3 ± 0.7
DBH of large trees ≥ 10 cm DBH	16.3 ± 2.8	13.5 ± 1.8^a	16.5 ± 4.8^b	16.0 ± 4.2^{ab}	18.7 ± 9.2
Distance of small trees 5-9.9 cm DBH	5.7 ± 2.1	2.4 ± 0.8	2.9 ± 1.3	3.0 ± 1.2	2.8 ± 0.4
DBH of small trees 5-9.9 cm DBH	8.5 ± 0.6	6.8 ± 0.5	6.9 ± 0.6	6.9 ± 0.8	7.5 ± 0.5
Density of large trees ≥ 10 cm DBH	399 ± 230	1326 ± 1052	1017 ± 698	1351 ± 1573	1039 ± 446
Density of small trees 5-9.9 cm DBH	482 ± 459	2186 ± 1271	1848 ± 1574	1541 ± 957	1330 ± 337
Density of trees ≥ 5 cm DBH	881 ± 612	3512 ± 1669	2865 ± 1761	2892 ± 2065	2369 ± 685

Table 3. Selectivity of *Microcebus* spp. for microhabitat structures. Significant deviations from 1 (= no selectivity) with $p < 0.05$ (based on one sample t-test) are marked in bold.

	Hazofotsy		Mangatsiaka		Ambatoabo & Ankoba
	<i>M. griseorufus</i>	<i>M. griseorufus</i>	<i>M. murinus</i>	Hybrids	<i>M. murinus</i>
N	9	21	65	28	6
Distance of large trees ≥ 10 cm DBH	1.04 ± 0.35	1.02 ± 0.37	1.16 ± 0.51	1.05 ± 0.40	1.06 ± 0.23
DBH of large trees ≥ 10 cm DBH	0.97 ± 0.17	0.93 ± 0.12	1.14 ± 0.33	1.10 ± 0.29	1.12 ± 0.55
Distance of small trees 5-9.9 cm DBH	1.00 ± 0.36	0.94 ± 0.30	1.13 ± 0.50	1.15 ± 0.47	1.13 ± 0.17
DBH of small trees 5-9.9 cm DBH	1.03 ± 0.07	1.00 ± 0.07	1.01 ± 0.09	1.02 ± 0.12	1.06 ± 0.07
Density of large trees ≥ 10 cm DBH	1.16 ± 0.67	1.43 ± 1.14	1.10 ± 0.75	1.46 ± 1.70	1.00 ± 0.43
Density of small trees 5-9.9 cm DBH	1.54 ± 1.47	1.48 ± 0.86	1.25 ± 1.06	1.04 ± 0.65	0.80 ± 0.20
Density of trees ≥ 5 cm DBH	1.32 ± 0.92	1.32 ± 0.63	1.08 ± 0.67	1.09 ± 0.78	0.81 ± 0.24

In Mangatsiaka, the microhabitats used by the two species differ only in the DBH of large trees (Table 2). Large trees used by *M. griseorufus* were smaller than those used by *M. murinus*. Hybrids between the two species used intermediate sized trees.

Discussion

Species with similar ecological requirements can only coexist if they differ in at least one niche dimension. In primates, possible competition seems to be aggravated between congeneric species (Houlé 1997). Interspecific differences in habitat utilization can allow species separation. This has been described for a number of possibly competing species especially in Africa and Asia (Schreier et al. 2009). In Madagascar, different modes of locomotion have been invoked as the actual reason for these differences, such as the need for vertical structures in case of leaping indriids and *Lepilemur* (e.g., Demes et al. 1995; Ganzhorn 1989; Warren and Crompton 1998). In case of species with less specialized modes of locomotion, the economic benefits of utilizing different or specific habitat structures remain unclear. These interspecific differences in habitat use could be a consequence of competitive exclusion (Thorén et al. 2010), though, if shifts in habitat utilization resemble systematic character displacements, they should be associated with specific energetic constraints, such as locomotion, and might have consequences for feeding behaviour (Fleagle 1984).

Differences in food composition, but also in habitat components have been invoked to allow species separation and therefore coexistence of similar sized mouse lemurs (Rendigs et al. 2003; Radespiel et al. 2003; Rakotondravony and Radespiel 2009; Thorén et al. 2011). The evolutionary benefit of one type of habitat utilization over the other remains enigmatic. In case of sister species, such as *Microcebus murinus* and *M. griseorufus* competition is expected to be even stronger due to their phylogenetic similarity (Houlé 1997). This is supported by the information available so far that suggests a strict separation of the two species based on habitat types (Yoder et al. 2002; Rasoazanabary 2004; Génin 2008; Gligor et al. 2009). Yet, the reason for the clear separation is unclear. *Microcebus murinus* occurs in a wide range of habitats with considerable phenotypic plasticity (Lahann et al. 2006). Similarly, *Microcebus griseorufus* occupies a wide variety of habitat types in regions where it is the only *Microcebus* species present (Rakotondranary et al. 2010). In allopatry, neither species shows any selectivity for specific habitat structures

(Andrianasolo et al. 2006; Rakotondranary et al. 2010). In the present study, this flexibility is reflected by the lack of habitat selection where the two species occur allopatrically. But, at sites, where the two species meet, they both deviate in their utilization of habitat structures from what is available in the habitat. In sympatry, microhabitats used by *Microcebus murinus*, *M. griseorufus* and hybrids differ in the diameter of large trees. *Microcebus griseorufus* occurs in forest patches with smaller diameters of large trees. In sympatry, the diameters of large trees used by *Microcebus murinus* and hybrids are larger compared to those used by *M. griseorufus*. Large trees might provide more holes for shelter than smaller trees and may be important for nest sites that, in case of *Microcebus*, allow the species to enter prolonged phases of reduced energy metabolism (Schmid and Ganzhorn 2009; Kobbe et al. 2011; Radespiel et al. 2003; Andrianasolo et al. 2006).

In contrast to the parent species, hybrids between *M. murinus* and *M. griseorufus* have broad habitat tolerances and do not show any deviation from the structures of the representative samples in the dry spiny forest where they are sympatric with both parent species. This flexibility of the hybrids is also reflected in their food composition where they are intermediate between the two parent species (Rakotondranary et al. submitted). These differences between the parent species, where they occur in sympatry, and the lack of habitat selectivity in hybrids from the same site indicate, that there are species-specific advantages associated with the utilization of specific habitat structures, though the evolutionary benefits are not understood. These subtle advantages could either have led to speciation at ecotones (Dewar and Richard 2007; Wilmé et al. 2006; Pearson and Raxworthy 2008; Vences et al. 2009) or they maintain species identities at secondary contact zones where species meet again after they have evolved distinct adaptations in allopatry. Thus, habitat separation seems to represent an important component for the evolution and maintenance of lemur species. As a next step we ought to search for the evolutionary advantage of those specific habitat components for species that are very similar otherwise.

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GENERAL DISCUSSION

Ecology aims at understanding the constraints and processes that determine the distribution and abundance of organisms (Krebs 2001). Ultimately, this is linked to the questions on how species evolve and how they maintain their species' identity. Over the last few decades, Madagascar has been one of the centres of interest to address these questions (Dewar and Richard 2007, Wilmé *et al.* 2006, Pearson and Raxworthy 2009, Vences *et al.* 2009). In this thesis, I use two sympatric sibling species of lemurs, *Microcebus murinus* and *M. griseorufus*, to study their distribution along a gradient of ecosystems and to determine the mechanisms that allow these two species to coexist despite seemingly identical ecological requirements.

1 Distribution of *Microcebus griseorufus*, *M. murinus* and their hybrids associated with environmental conditions (Chapter 1)

Abilities to use a resource under varying environmental conditions are one form of resource partitioning that allows competing species to occur in the same area. *Microcebus murinus* and *M. griseorufus* show clear associations with specific habitat types that are related to the different abiotic and biotic environmental conditions. Along the vegetation gradient of Andohahela National Park, *M. griseorufus* is associated with dry conditions, while *M. murinus* is associated with more mesic conditions. The associations of the two species with respect to environmental conditions match the pattern found at the other site (Yoder *et al.* 2002, Génin 2008). These types of associations might be linked to the abilities to use a resource under varying environmental conditions. Though *M. murinus* is the most widespread mouse lemur species in Madagascar and living in different vegetation formations, the species is absent from Hazofotsy where higher temperature and lower humidity prevail than in the other sites at Andohahela National Park. This absence could be explained by competitive exclusion of *M. murinus* by *M. griseorufus*. As the latter species shows very high physiological flexibilities with respect to energy saving strategies that have not been described for other species yet (Kobbe *et al.* 2010). *Microcebus griseorufus*, in turn, seem to be excluded from mesic vegetation formation such as the gallery forest by *M.*

murinus. *Microcebus murinus* and *M. griseorufus* co-occur and hybridize in the dry vegetation formations at the interface of mesic and dry habitats (Gligor *et al.* 2009, Hapke *et al.* submitted).

According to Hutchinson (1959), differences in body mass are important to allow the coexistence of congeneric species. This explanation does not apply to *M. murinus* and *M. griseorufus*. The two species are of almost identical size and therefore differences in body mass or other morphological characteristics between *M. griseorufus* and *M. murinus* do not contribute to species separation (Chapter 1).

Apart from interspecific differences in the mean values of morphological characters, morphological measurements can vary gradually within a species in relation to environmental conditions, reflecting adaptations to ambient temperatures and resource abundance. Lower body mass is considered an adaptation to increased average ambient temperatures as predicted by Bergmann's rule. While this idea is supported by a large number of studies (reviewed by McNab 2010) and has also been found in lemurs (Albrecht *et al.* 1990; Ravosa *et al.* 1993; Godfrey *et al.* 1999; Lehman *et al.* 2005) including *M. murinus* (Lahann *et al.* 2006), different ambient conditions are not reflected in changes in body measurements of *M. murinus* living in allopatry in the different vegetation formations of Andohahela National Park. Thus, at the geographical scale considered in the present thesis, morphological measures do not seem to represent traits that vary in relation to environmental conditions or contribute to species separation. Thus, competition ought to be reduced by other forms of niche partitioning.

2 Separation related to food composition and microhabitat utilization (Chapters 2 and 3)

Food and space are among the most important axes for niche partitioning (Schoener 1974). Several studies on food and habitat partitioning among sympatric mouse lemurs have been conducted to understand the mechanism allowing their co-occurrence. Examples are presented in Table 1. These studies have demonstrated that separation between *Microcebus murinus* and *M. ravelobensis* in sympatry is achieved through a combination of differences in food composition and the reduction of energy requirements in *M. murinus* during the lean season (Thorén *et al.* 2011b). Separation of sympatric *M. berthae* and *M. murinus* is achieved through differences in body mass, spatial exclusion on the level of microhabitats and difference in the composition and seasonal variation of their diets (Schwab and Ganzhorn 2004, Dammhahn and Kappeler 2008).

Table 1. Studies on species separation of sympatric *Microcebus* species in Madagascar; body mass and head-body length according to Mittermeier *et al.* (2006).

	Western	Northwestern	Southeastern
Study site	Kirindy	Ampijoroa, Ankarafantsika	Andohahela National Parks
Species	<i>M. murinus</i>	<i>M. murinus</i>	<i>M. murinus</i>
(Body mass;	(58-67g; 12-14cm)	(58-67g; 12-14cm)	(58-67g; 12-14cm)
head-body	<i>M. berthae</i>	<i>M. ravelobensis</i>	<i>M. griseorufus</i>
length)	(30g; 9-9.5 cm)	(56-87g; 12-13cm)	(46-79g; 12-13cm)
Size difference	Yes	No	No
Forest type	Dry deciduous forest	Dry deciduous forest	Dry spiny forest
Methods used on feeding analyses	Focal observation Faecal analyses Isotope (¹⁵ N, ¹³ C) analyses	Focal observation Faecal analyses	Faecal analyses Isotope (¹⁵ N, ¹³ C) analyses
Resource Partitioning	Habitat utilization and food composition.	Habitat utilization and food composition; reduction in locomotor activity over limited resource	Habitat utilization and food composition
References	Schwab and Ganzhorn 2004, Dammhahn and Kappeler 2008, 2010	Rakotondravony and Radespiel 2009, Thorén <i>et al.</i> 2011a,b	This study (Chapters 2 and 3)

Chapter 2 illustrates the power but also the pitfalls of stable isotope analyses for the study of dietary patterns and trophic relationships within ecosystems. In our analyses, *Microcebus murinus* living in allopatry, differed substantially in their isotope signatures (¹⁵N and ¹³C) between different types of vegetation that occurred in close proximity. This difference seems to be more due to the ambient conditions rather than due to differences in trophic levels. Thus, comparisons of isotope studies from species living in different habitats must be controlled for the baseline isotope signatures of the food items. This confounding variable is not a problem, when comparing two species from the same site, as done in our study where we compared the two species in sympatry.

According to the present study, separation of *Microcebus murinus* and *M. griseorufus* co-occurring in the same area is achieved through different degrees of insectivory during the dry season. During this season of food scarcity, *M. griseorufus* ingest more animal matter (insects) than *M. murinus*. During the wet season, when food is abundant, however, the two species do not differ in food composition. The high consumption of insect matter matches the patterns found in

other communities where a second mouse lemur species lives in sympatry with *Microcebus murinus*. *Microcebus ravelobensis* (in the northwest) and *M. berthae* (in the west) also feed more on insect secretion than *M. murinus* (Dammhahn and Kappeler 2008, Thorén 2011a).

Separation between sympatric mouse lemurs could be also achieved by energy saving strategies. In the other two systems, where *Microcebus murinus* coexists with a second species of *Microcebus*, *M. murinus* is more likely to use energy saving strategies than the other species (Schmid and Speakman 2000, Schmid *et al.* 2000, Radespiel *et al.* 2003, Thorén *et al.* 2011a,b). In the system where *M. murinus* co-occurs with *M. griseorufus*, the situation appears to be reversed. During periods of low ambient temperature and food scarcity, *M. murinus* as well as *M. griseorufus* can enter torpor and hibernation (Kobbe and Dausmann 2009, Schmid and Ganzhorn 2009, Kobbe *et al.* 2011). Difference in microhabitat utilization between *M. murinus* and *M. griseorufus* could then be explained as a consequence (or prerequisite) for different energy saving strategies, such as torpor and hibernation. In Andohahela National Park, *Microcebus murinus* was found in microhabitats with larger trees than average while *M. griseorufus* utilized microhabitats with smaller trees than average (Chapter 3). Large trees might provide more holes for shelter than smaller trees and might be important for nest sites that, in case of *Microcebus*, allow the species to enter prolonged phases of reduced energy metabolism (Chapter 3).

To summarize, *Microcebus murinus* and *M. griseorufus* are associated with different environmental conditions. Co-occurrence of these congeneric species is achieved through microhabitat differentiation and different degrees of insectivory during the lean season. *Microcebus griseorufus* eat more insects than *M. murinus*. *Microcebus murinus* was found in microhabitats with larger trees than average while *M. griseorufus* utilized microhabitats with smaller trees than average. Thus, the two *Microcebus* species studied here seem to apply similar mechanisms for species separation as the other species pairs of *Microcebus* studied previously. Subsequent studies might want to focus on the role of different time and energy saving strategies that are linked to different food and habitat utilization patterns, but that are not fully explored yet.

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SUMMARY

Classical hypotheses suggest that two species cannot coexist if they share the same ecological niche. Observations of a number of species sharing the same habitat suggest that species can coexist if they utilize different resources, adapt differently to environmental conditions, or differ in size.

In this thesis, I use two sympatric species of mouse lemurs, the reddish-gray mouse lemur (*Microcebus griseorufus*) and the gray mouse lemur (*M. murinus*), to study their distribution along a gradient of ecosystems and to determine the mechanisms that allow these two species to coexist despite seemingly identical ecological requirements. These two species are morphologically very similar and represent sister species, i.e., they are the least likely primate species to coexist due to their phylogenetic similarity. They co-occur in the dry spiny forest in southern Madagascar.

This study was carried out from September 2006 to June 2009 at Andohahela National Park in the extreme south-east of Madagascar. This area provides an ideal opportunity to investigate the various patterns and evolutionary constraints along a continuous environmental gradient ranging from evergreen humid rainforest to dry spiny forest.

The aims of this study were (1) to investigate the environmental conditions that are associated with the distribution of the two *Microcebus* species and their hybrids; (2) to study the possible mechanisms that allow the coexistence of these sympatric congeneric species (*M. murinus* and *M. griseorufus*) by investigating the potential separation with respect to food composition. This study is based on stable isotopes as an indirect measure of the trophic level of the consumer and its prey; (3) to describe microhabitat utilization of both species.

The results showed that *Microcebus murinus* and *M. griseorufus* are clearly associated with specific habitat types that are related to the different abiotic and biotic environmental conditions. Along the vegetation gradient of Andohahela National Park, *M. griseorufus* is associated with dry conditions, while *M. murinus* is associated with more mesic conditions. Co-occurrence of these congeneric species appears to be allowed through microhabitat differentiation and different degrees of insectivory, during the lean season, *M. griseorufus* eat more insects than *M. murinus*. *Microcebus murinus* was found in microhabitats with larger trees than average while *M. griseorufus* utilized microhabitats with smaller trees than average.

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