Exploring niche partioning in strepsirrhines: *Lepilemur fleuretae* and *Avahi meridionalis* in the lowland rainforest of south-eastern Madagascar

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1. Summary of the project proposal

Introduction

Mechanisms of niche partitioning among lemurs are still poorly understood and there is an intense debate concerning the manner in which these primates diversified to an extent unmatched by any other primate radiation (1). The sympatric lemur genera *Lepilemur* and *Avahi* are ideal models to study fine-grained mechanisms of niche partitioning in lemurs, since these strepsirrhines are ecologically similar because both are nocturnal, folivorous, and have comparable body weight (2). These traits are exceptional among primates for two main reasons. First, nocturnality is unusual among arboreal folivores, since nocturnal, arboreal mammals tend to be small and their metabolic constraints do not allow them to survive on a low-energy diet (3). In fact, both *Avahi* and *Lepilemur* have a relatively tiny body mass (ranging from 700 to 1400 g), which is at the lower limits for folivory in primates (4). Second, folivory at night is rare, since color vision is important to discern leaves with higher protein content (5).

Despite these similarities, comparative studies conducted so far found limited niche overlap between *L. edwardsi* and *A. occidentalis* in Malagasy deciduous forests. This has been linked to the differences between the two genera in terms of food selection and social structure (2,6). However, the above mechanisms do not always seem to work. In fact, it has been shown that *Lepilemur* has a lower diet quality when in sympatry with *Avahi* (7), and the interaction between the two taxa may have led to competitive exclusion in other habitats on the island. Unfortunately, little is known about the niche partitioning of the two genera in the main habitat where they occur simpatrically, the rain-forest.

Recent advances in field technology allow investigation of niche partitioning with a more powerful lens. For instance, the use of auto-logging accelerometers to record 24-hour activity patterns will increase exponentially our resolution in terms of temporal niche overlap. Also, evaluation of food intake with continuous sampling has not been used in previous studies on these two genera despite its being commonly used to understand metabolized energy in primates (8). Finally, the use of skin-temperature tags has the potential to establish if the two species use physiological adaptations to reduce energy consumption (9,10). The information collected with this new approach has a great potential to extend our understanding of the finely tuned ecological factors that have allowed the successful co-habitation of *Avahi* and *Lepilemur*.

The main aim of this study was to assess the ecological niche partitioning in *Lepilemur fleuretae* (Fig. 1) and *Avahi meridionalis* (Fig. 2) in the Tsitongambarika (TGK) Protected Area, South-Eastern Madagascar. Considering that this area comprises one of the largest expansions of primary lowland rainforest on the island it represents an ideal site on which to study the sympatry between the two taxa in a humid forest. Interestingly, TGK is contiguous to the littoral forests on sandy soil, where *Lepilemur* does not occur while *Avahi* is abundant (11). Thus, by comparing our results with previous data on *Avahi* in littoral forests (11), our data may help to understand whether the absence of *Lepilemur* in certain areas may be caused by competitive exclusion.

The second aim of this study was to set-up field research in the area of TGK, which is among the sites that have been prioritized for research and conservation in the 2013-2016 Lemur Conservation Action Plan (12). *Lepilemur fleuretae* and *Avahi meridionalis*, in particular, have been identified as priority species for research, being almost unknown to science and yet listed as Critically Endangered and Endangered respectively.

Figure 1 *Lepilemur fleuretae*. Photo by George Selly

By this research, in particular, we wanted to assess whether *Lepilemur* and *Avahi* show differences in the three main niche dimensions that have been shown to reduce ecological competition: habitat, time, and food (13). According to the "niche complementarity hypothesis", a high overlap in one niche dimension should be compensated by a low overlap in at least one of the other dimensions (14). Specifically, we planned to:

i) Measure activity patterns via high-resolution accelerometers to assess whether a temporal niche partitioning occurs. *Avahi* is considered a secondarily nocturnal species (15) while *Lepilemur* has nocturnal ancestors. We thus hypothesize that *Avahi* has retained more crepuscular activity.

ii) Measure nutrient intake by recording food intake and performing nutritional analysis to increase the resolution on dietary differences. *Avahi* has an allele that performs better in distinguishing young leaves unlike the allele present in *Lepilemur* (16). We thus hypothesize that there is a higher-quality and selective diet in *Avahi*.

iii) Determine differences in habitat requirements by recording ranging pattern and species-specific use of canopy. Due to the secondary nocturnality in *Avahi* and the different selection for dichromacy between the two genera, we expect *Avahi* to use higher, brighter strata of the forest.

iv) Measure skin temperature via data-loggers attached to radio-collars to assess whether the two species use heterothermy. *Lepilemur ruficaudatus* has been shown to possess the lowest metabolic rates among mammals (17). We thus hypothesize that *Lepilemur* may use heterothermy to preserve energy, as seen in other small nocturnal lemur species (9,10).

Figure 2 *Avahi meridionalis*. Photo by George Selly

Methods

We conducted the study at the Ampasy Biological Station (24°34'S, 47°09'E), in the Northernmost portion of TGK (region of Fort Dauphin). Data collection took place over a 14-month period. Below what we planned and what we actually did:

1. Perform population surveys during the initial two months via distance sampling using 15 independent transects (1-km length)(18).

We actually performed surveys for the whole study period walking 9 independent transects. This in order to get densities of animals and not just encounter rates. This way, we might increase our chances of publishing due to a higher sample size.

2. Capture a total of 15 individuals (10 individuals of *Lepilemur fleuretae* and 5 individuals belonging to five different pair groups of *Avahi meridionalis*) via the aid of a team of experts.

We actually reduced the number of animals captured to 6 *Lepilemur* and 6 *Avahi*. We reduced the number since observing 20 animals would have been too time consuming. We captured an even number of *Avahi* and *Lepilemur* since, contrary to what expected, many individuals of *Avahi* were found alone and not in pairs.

3. Collect behavioral data on each focal animal for 6 hours (from 6.00 p.m. to 0.00 a.m. or from 0.00 a.m. to 6.00 a.m.) a month over a one-year period. We planned to use the Instantaneous Focal Sampling, with records at 5-minute intervals, coupled with the All Occurrences (19). To assess the species-specific use of canopy, we planned to collect also the animal height every 5 minutes, grouping them into 5 strata: 0-5m, 6-10m, 11-15 m, 16-20 m, and >20m (20).

We actually collected data via Continuous Sampling in order to get as many data as possible due to the difficulties to do observations in this type of forest. We collected data on: activity, inclination and diameter of the support in which the animal was whilst feeding or resting, animal height, and proximity to other animals.

4. Collect feeding data by using the Continuous Focal Sampling (19). We also planned to record the food species and the food items (2, 11). We also planned to record food intake rates by using 1-min sessions within feeding bouts, recording the number of units consumed (8).

We collected feeding data as planned in the proposal.

5. Collect the animal location at 30-minute intervals via a handheld GPS.

We actually collected the animal location every hour during observations. Also, we collected ranging data every hour via triangulation (two complete nights per month on each animal) to increase the sample size.

6. Tag five individuals per plant species with diameter at breast high (DBH) greater than 10 cm (21,22) within 5 m of each side of two 1-km trails to estimate the potential food availability during the study period. Trees will be checked for the presence/absence of new leaves, flowers and unripe/ripe fruits twice a month (23).

We established the phenological trails as planned although we managed to check the presence/absence once a month and not twice due to the high number of trees in the trails (784).

7. Establish 10 random plots (10 m x 10 m) to characterize forest structure within the home range of each animal. In each plot, all the trees larger than 10 cm Diameter at Breast Height (DBH) will be measured and tagged (24).

We actually characterized the forest via 34 plots of 100 m x 10 m established along the transects (with 200m gaps in between).

8. Collect food samples of all the food items eaten by study groups as described by Norscia et al. (11). Nutritional analyses of food items will be performed at the Department of Animal Ecology and Conservation of Hamburg University via the methodology described by Ortmann et al. (25).

We collected food samples as planned.

9. Register temperature and relative humidity at 30-minute intervals via data loggers and measure rainfall daily. These data will be used to determine climatic seasons.

We collected temperature and humidity data as planned.

2. Brief summary of field activities

The data collection started the 2nd of May 2015 and finished the 14th of July 2016. We collected data via behavioural observations from August 2015 to July 2016 (by using continuous sampling method) on activity, diet, spatial use, sleeping sites, and ranging patterns (the data on ranging patterns had been also collected via triangulation to increase the sample size). Also, we collected data on phenology from July 2015 to July 2016, this data collection will be continued by a PhD student that is now at the camp to conduct her research. For the all period we collected data on lemur densities, which were necessary first to locate the animals for captures, and then to estimate the densities of animals and the ecological predictors. We collected ecological data via plots along the transects and this data will be shared with two Master students. Furthermore, we collected data on the hunting pressure in the area via questionnaires on 74 households from 10 villages close to the research station. Regarding the conservation education project, we performed training days to teachers belonging to schools from the community of Iaboakoho (the nearest village to the research station) once a month in July, August, September, and October. Furthermore, we also had activities with children, in collaboration with Asity Madagascar and Qit Madagascar Minerals, for the World Lemur Day the 31st of October. In July 2016 we asked 10 teachers from Iaboakoho to do a questionnaire of 20 structured questions in order to evaluate whether the information given have been retained. We also asked other 32 teachers from other three municipalities to do the test in order to check their level of knowledge on lemurs and their environment. We obtained the ethical clearance for the tests. The field station is now self-managed and new structures were built in December and April with the financial aid of Qit Madagascar Minerals and Oxford Brookes University. The plan is to have a permanent research station to increase the protection of the area.

3. Statement of objectives accomplished

The objectives accomplished during the study period are:

i. Capture 6 individuals per species to put VHF collars to allow systematic observations. Moreover, 4 individuals per species had been equipped with accelerometers and temperature tags.
ii. Collect behavioural data (activity, diet, ranging patterns) between August 2015 and July 2016.
iii. Increase the infrastructures at the research station (kitchen, 4 shelters, solar panel, and satellite phone).
iv. Collect phenological data once a month on 784 trees belonging to 208 species from July 2015 to July 2016 (the data collection will be continued by the next students).
v. Collect data on lemur densities by walking 9 different transects. A total of 119.0 km and 104.5 km had been walked during day and night respectively.
vi. Do four training days for teachers in the community where the research area is located. Also, a special event was organized for children during the World Lemur Day. At the end of the study, we performed a test to evaluate whether teachers retained the information given during the lessons. Moreover, teachers from other municipalities performed the same test to consider them as the control groups.

vii. Do households interviews with 74 people from 10 villages surrounding the research station in order to evaluate their use of the forest and the hunting pressure in the area. We obtained the ethical clearance for the interviews.

4. Results

After performing transects over 14 months to determine primate densities, we walked a total of 119.0 km during the day and 104.5 km at night. At the end of the data collection we will analyze the data via Distance software. Below we present encounter rates of the animals and groups evaluated via 9 transects performed around once a month by daytime and once a month by night-time (Table 1). After the data analysis with Distance a paper on this topic will be submitted for publication on the American Journal of Primatology or on the International Journal of Primatology.

Species	Encounter rate (animals/km)	Encounter rate (groups/km)
Avahi meridionalis	0.93	0.62
Cheirogaleus major	0.11	0.11
Daubentonia madagascariensis	0.02	0.02
Eulemur collaris	1.26	0.27
Hapalemur meridionalis	0.37	0.20
Lepilemur fleuretae	2.01	1.84
Microcebus tanosi	1.45	1.25

Table 1. Encounter rates of lemurs present at the forest close to the Ampasy research station.

In the 12 months of behavioural data collection, we collected 148 observation hours on *Avahi meridionalis* and 139 hours on *Lepilemur fleuretae*. The data had been collected via continuous sampling and overall percentages of behaviors are shown in table 2. Percentages of out of sight are 26.1% for *Avahi meridionalis* and 32.9% for *Lepilemur fleuretae*. More precise estimations of activity patterns will be available by analysing the data collected via the accelerometers (which also have a temperature tag). We collected 110 different feeding trees belonging to 39 species for *A. meridionalis* and 92 feeding trees belonging to 25 species for *L. fleuretae*. A total of 15 tree species are in common between the study species. We collected and dried all the food items eaten during the study period. We collected the food intake for 17 food items out of 51 and we plan to extrapolate a curve “food intake/leaf weight” to estimate the food intake for the food items we did not collect (of which we have the leaf weight). From a preliminary analysis it resulted that *Avahi meridionalis* is strictly folivorous, whilst *Lepilemur fleuretae* is mainly folivorous (80.2% of leaves in the diet) but integrated his diet with flowers (10.4%), fruits (9.3%), and insects (0.1%). For this part of ty research we plan to submit at least two papers, one focused on activity patterns evaluated via accelerometers and one evaluating the diet and the food intake.

	Avahi meridionalis	*Lepilemur fleuretae*
Resting	59.1	56.1
Moving	6.6	9.6
Feeding	26.7	29.6
Grooming	1.4	2.4
Social	6.2	2.3

Table 2. Percentages of time for each behavior for *Avahi meridionalis* and *Lepilemur fleuretae*

We collected a total of 24 sleeping sites for *Avahi meridionalis* and 32 for *Lepilemur fleuretae*. Apart from *Brochoneura acumita* that accounted for 28% of the sleeping sites, *Avahi meridionalis* didn't seem to prefer other tree species for sleeping. On the other hand, *Lepilemur fleuretae* had some preferred trees, namely *Syzigium* sp. (34.4%), *Viguieranthus alternans* (31.3%), and *Albizia gummifera* (15.6%). Further analyses will be performed and a paper on this topic will be submitted for publication on the American Journal of Primatology or the International Journal of Primatology.

We collected around 380 GPS point per each animal and I will calculate ranging patterns via Ranges 8 software and a paper on this topic will be submitted for publication to the American Journal of Primatology or the International Journal of Primatology. The GPS points resulted from both triangulation and direct observations and were taken every hour.

Phenological data had been taken once a month from July 2015 to July 2016. The overall analysis is not ready yet, but, when ready, it will be submitted for publication on Biotropica.

Hunting pressure on lemurs has not been reported in the area since the installation of the research station. This is a good indicator that the presence of researchers, with the creation of new job opportunities, is an effective way to decrease human pressure on the forest. Also, densities of *Hapalemur meridionalis* and *Eulemur collaris* increased in the second part of the study by considering the data obtained performing the transects (although a statistical test is necessary), probably meaning that the hunting pressure in the area is decreased after the installation of the research station. To evaluate the impact of hunting in the villages, we further performed household interviews in ten villages close to the research station (no more than 8 km far) for a total of 74 interviews. Data from interviews are aimed to be published on Oryx.

We also asked teachers from the four municipalities close to the Tsitongambarika forest to test their knowledge on lemurs and their environment. In total, 42 teachers filled the questionnaires. The preliminary analysis suggested that teachers from Iaboahako who received the training around one year before, gave a higher percentage of right answers (median: 81.6%, quartiles: 64.5-84.2%) than the teachers from the other municipalities (Ampasy-Nahampoana: 47.4%, 42.1-63.2%; Mandromondromontra: 47.4%, 38.2-51.3%; Mahatalaky: 47.4%, 36.8-60.5%). The data from the questionnaires will be submitted for publication on Lemur News or peer-reviewed journals if accepted.

Another aim of the project was to test whether the sportive lemur present in Tsitongambarika is *Lepilemur fleuretae* or another species. The genetic analyses are not ready yet and will be shared once available.

5. Discussion

Training and educational accomplishments.

Two volunteers came from July to September 2015 and other two came from April to July 2016. They were trained to perform data collection and helping during the data collection in general. Moreover, a Malagasy MSc student collaborated in collecting data regarding the phenology (e.g. species identification). I delivered four presentations to local teachers from four villages nearby the research station (one each month from September to October 2015) to give information about the importance of biodiversity, animals, and the forest. This is particularly important to raise awareness in local communities, starting from children. I performed these training days with the collaboration of Asity Madagascar that is responsible of the management of the area. The lessons had been retained as shown by the preliminary results (see below) and it can be a first indication that a program of environmental education might be helpful in the area. Also, we organized a special event for children for the World Lemur Day the 31st of October. The event was successful and attracted many people from the villages. We organized several games for children involving questions about the forest and lemurs. The training given to the two local guides was successful and they are now able to work with researchers, helping on behavioural observations and plant identification.

Research accomplishments.

The research was overall successful, although problems emerged due to the difficulties of the study area. We collected roughly the same observation hours taken by other studies (2, 6, which compared sportive and wholly lemurs. No manuscripts have been submitted for publication yet, since the data collection finished on the 14th of July 2016. Considering the amount of data collected, we are confident that we have enough data to submit at least four publications in peer-reviewed journals.

Problems and assistance needed.

Data collection had been difficult from the beginning of the rainy season since the area is particularly wet. As a consequence, the increasing of water level in rivers nearby the research station created problems in reaching the observation areas. This limited the observation hours in December and January. Three collared individuals, one *Avahi* and two *Lepilemur*, were killed by the local predator, fosa, all of them were equipped with accelerometers. In March, we tried to capture other individuals to replace the dead animals, but the capture team was specialized in blowpipe and it was not feasible to catch them with this method. Thus, to recapture the lemurs in July we asked the team of Ed Louis, equipped with a dart gun, to come back.

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