

RESEARCH ARTICLE

Activity patterns in seven captive lemur species: Evidence of cathemerality in *Varecia* and *Lemur catta*?Joel Bray¹  | David R. Samson² | Charles L. Nunn^{2,3}¹School of Human Evolution and Social Change, Arizona State University, Tempe, Arizona²Department of Evolutionary Anthropology, Duke University, Durham, North Carolina³Duke Global Health Institute, Duke University, Durham, North Carolina**Correspondence**Joel Bray, School of Human Evolution and Social Change, Arizona State University, P.O. Box 872402, Tempe, AZ 85287.
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Cathemerality, or activity throughout the 24-hr cycle, is rare in primates yet relatively common among lemurs. However, the diverse ecological conditions under which cathemerality is expressed complicates attempts to identify species-typical behavior. For example, *Lemur catta* and *Varecia* have historically been described as diurnal, yet recent studies suggest that they might exhibit cathemeral behavior under some conditions. To investigate this variation, we monitored activity patterns among lemurs that are exposed to similar captive environments. Using MotionWatch 8 @ actigraphy data loggers, we studied 88 lemurs across seven species at the Duke Lemur Center (DLC). Six species were members of the family Lemuridae (*Eulemur coronatus*, *E. flavifrons*, *E. mongoz*, *L. catta*, *V. rubra*, *V. variegata*), while a seventh was strictly diurnal and included as an out-group (*Propithecus coquereli*). For each 24-hr cycle ($N = 503$), we generated two estimates of cathemerality: mean night (MN) activity and day/night (DN) activity ratio (day and night cutoffs were based on astronomical twilights). As expected, *P. coquereli* engaged in the least amount of nocturnal activity according to both measures; their activity was also outside the 95% confidence intervals of all three cathemeral *Eulemur* species, which exhibited the greatest evidence of cathemerality. By these estimates, *Varecia* activity was most similar to *Eulemur* and exhibited substantial deviations from *P. coquereli* (β (MN) = $0.22 \pm SE$ 0.12; β (DN) = $-0.21 \pm SE$ 0.12). *L. catta* activity patterns also deviated from *P. coquereli* (β (MN) = $0.12 \pm SE$ 0.11; β (DN) = $-0.15 \pm SE$ 0.12) but to a lesser degree than either *Varecia* or *Eulemur*. Overall, *L. catta* displayed an intermediate activity pattern between *Eulemur* and *P. coquereli*, which is somewhat consistent with wild studies. Regarding *Varecia*, although additional observations in more diverse wild habitats are needed, our findings support the existence of cathemeral behavior in this genus.

KEYWORDS

actigraphy, circadian rhythm, diurnal, nocturnal, prosimian

1 | INTRODUCTION

Most mammals exhibit a suite of morphological, physiological, and behavioral adaptations for either diurnal or nocturnal activity (Ashby, 1972; Halle, 2006; Halle & Stenseth, 2000). Among primates, diurnal activity patterns characterize almost all haplorhines, while nocturnal activity patterns characterize most strepsirhines (Santini, Rojas, & Donati, 2015). Some species, however, exhibit a flexible activity pattern that is defined by substantial activity throughout the light and dark portions of the 24-hr cycle (Halle, 2006; Tattersall, 1987). This

behavioral pattern, known as cathemerality, is well documented among species of the family Lemuridae (reviewed in Curtis & Rasmussen, 2006; Donati & Borgognini-Tarli, 2006a).

The evolution of cathemerality in lemurs has been linked to Madagascar's climatic unpredictability (Donati & Borgognini-Tarli, 2006a; Wright, 1999) and hyper-variable patterns of precipitation (Dewar & Richard, 2007). Specifically, cathemeral behavior is hypothesized to provide one or more ecological advantages, including thermoregulatory benefits to avoid both extreme heat and extreme cold (Curtis, Zaramody, & Martin, 1999; LaFleur et al., 2014;

Mutschler, 2002), reduced predation risk (reviewed in Colquhoun, 2006, 2007; Rasmussen, 2005), reduced competition from sympatric species (Curtis et al., 1999; Rasmussen, 1999), and increased metabolic efficiency related to a fiber-rich diet (Donati, Baldi, Morelli, Ganzhorn, & Borgognini-Tarli, 2009; Donati, Bollen, Borgognini-Tarli, & Ganzhorn, 2007; Engqvist & Richard, 1991). Alternatively, the evolutionary disequilibrium hypothesis posits that cathemeral behavior represents a transitional state between nocturnal and diurnal activity patterns brought about by the extinction of large predators and other lemurs upon the arrival of humans to Madagascar (Kappeler & Erkert, 2003; van Schaik & Kappeler, 1993, 1996). Reconstructions of cathemerality on deep nodes of the lemur phylogeny, however, support an ancient and adaptive origin (Griffin, Matthews, & Nunn, 2012; Santini et al., 2015).

Determining which lemur species express cathemeral behavior is critical to testing hypotheses regarding its evolution (Donati & Borgognini-Tarli, 2006a; Donati, Santini, Razafindramanana, Boitani, & Borgognini-Tarli, 2013). However, attempts to identify species-typical activity patterns are complicated by environmental and seasonal factors that mask endogenous circadian rhythms. In lemurs, masking factors include day length, temperature, moonlight, and food availability (Curtis et al., 1999; Donati et al., 2009; Eppley, Ganzhorn, & Donati, 2015; reviewed in Curtis & Rasmussen, 2002; Donati & Borgognini-Tarli, 2006a). This environmental variation may explain differences in activity patterns among populations of the same species. For example, in a study in Berenty Reserve, Donati et al. (2013) recorded the activity of *Lemur catta* across two different forest types and found that while lemurs in one population were diurnal (consistent with previous observations of the species), lemurs in the second population were cathemeral.

Comparative studies in captivity represent one method of addressing these challenges. By controlling for environmental variables that are known to influence activity patterns, this approach can reveal whether endogenous circadian rhythms or exogenous environmental factors explain observed differences between species that have only been studied in distinct ecological environments in the wild. For instance, variation in the activity patterns of two captive species may indicate differences in their endogenous circadian rhythms (Santini-Palka, 1994). Alternatively, similar activity patterns in captivity between two species reported to have different activity patterns in the wild might suggest that exogenous environmental factors in the wild mask a shared circadian rhythm. Such a finding would suggest that these species might exhibit similar activity patterns in the wild if exposed to similar environmental conditions.

Historically, the family Lemuridae (Figure 1) has contained both diurnal species, such as *Varecia variegata*, *V. rubra*, and *L. catta*, and cathemeral species, including all species of *Eulemur* (Curtis & Rasmussen, 2002). As noted above, however, a recent study discovered cathemeral behavior in wild *L. catta* (Donati et al., 2013), with similar findings in another study (LaFleur et al., 2014; see also Parga, 2011). *Varecia* has formally been described as diurnal (Vasey, 2005), but reviews of cathemerality reference anecdotal reports of cathemeral behavior in wild *V. variegata* (Donati & Borgognini-Tarli, 2006a; Wright, 1999).

In a recent study at the Duke Lemur Center (DLC), Rea, Figueiro, Jones, and Glander (2014) investigated the influence of light on lemur

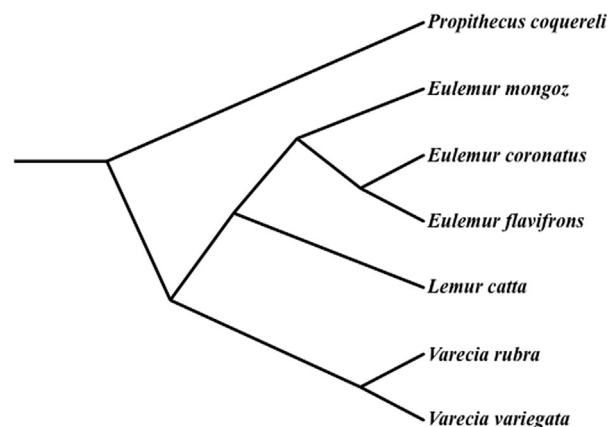


FIGURE 1 Phylogenetic relationships among species in this study. The family Lemuridae includes all species listed except for *P. coquereli*. Phylogeny is based on Markolf and Kappeler (2013)

activity-rest patterns in five species (*E. mongoz*, *L. catta*, *V. rubra*, *V. variegata*, and *Propithecus coquereli*). Using a Daysimeter-D (Lighting Research Center, Troy, New York) to measure both light and activity simultaneously, all species exhibited considerable variation in activity across the 24-hr light cycle. All species also displayed an anticipatory increase in activity before sunrise. In addition to these primary findings, the study presented some intriguing results regarding species-specific activity patterns. Specifically, the authors found a lower day/night activity ratio (a measure of cathemerality that compares the amount of activity expressed during the day vs. at night) in *V. rubra* and *V. variegata* than in *E. mongoz*. Furthermore, among these captive populations, *L. catta* actually had a relatively high day/night activity ratio, which would not be expected with cathemerality. The number of subjects per species in this study was small (range: 2–4), however, making it difficult to know whether these activity patterns are representative of the captive population as a whole. The *L. catta* population was also monitored only during the short nights of summer, warranting investigation during other seasons. Additionally, the activity ratios were based on astronomical sunrise and sunset, not astronomical twilights, which may produce biased estimates of nocturnal activity (Donati et al., 2013; Fernández-Duque, de la Iglesia, & Erkert, 2010).

To further investigate lemur activity patterns, we used actigraphy to collect data on seven species at the DLC. The captive setting meant that species could be monitored in relation to abiotic factors, such as day length, in otherwise similar ecological and environmental conditions. We included the five species studied by Rea et al. (2014) and two additional species, *E. coronatus* and *E. flavifrons*. Although *P. coquereli* is not a member of the family Lemuridae, evidence from captivity (Rea et al., 2014) and a closely related species in the wild (*P. verreauxi*; Erkert & Kappeler, 2004) strongly suggest that it is strictly diurnal; it was thus included in this study as both an out-group and control. *Haplemur*, which is also cathemeral (Mutschler, Feistner, & Nievergelt, 1998) and a member of Lemuridae was not included in the study because only three subjects were housed at the DLC at the time of the study.

The captive environment shifts cathemeral lemurs to a generally diurnal pattern due to human activity during the day (Rea et al., 2014); nonetheless, we hypothesized that cathemeral and diurnal species would show variation in the amount of activity exhibited across the 24-hr cycle, attributable to distinct endogenous circadian rhythms. Assuming that this first hypothesis would be supported, we further hypothesized that cathemeral species would exhibit more nocturnal activity than diurnal species. Unlike previous work with this population, we directly compared the activity of *L. catta* and both *Varecia* species to the activity of *P. coquereli* and three *Eulemur* species to quantify the degree to which their activity was similar to or different from well-established diurnal and cathemeral primates. Activity patterns similar to *Propithecus* were interpreted as more diurnal, while activity patterns similar to the *Eulemur* species were interpreted as more cathemeral.

2 | METHODS

All animal use and methods were approved by the Duke University Institutional Animal Care and Use Committee (Protocol #: A236-13-09) and the DLC Research Committee. The research also adhered to legal requirements and to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

2.1 | Study site and animals

We studied the activity patterns of seven lemur species: crowned lemurs (*E. coronatus*), blue-eyed black lemurs (*E. flavifrons*), mongoose lemurs (*E. mongoz*), ring-tailed lemurs (*L. catta*), red-ruffed lemurs (*V. rubra*), black-and-white ruffed lemurs (*V. variegata*), and Coquerel's sifakas (*Propithecus coquereli*) (Table 1). All subjects were housed at the DLC in Durham, North Carolina (N 35° 59' 35", W 78° 57' 38"). The DLC is located within Duke Forest, a rural and wooded expanse. During the study period (January–July 2014), temperatures ranged from −13.7°C to 37.5°C (mean = 14.5°C), humidity ranged between 18% and 100% (mean = 71.4%), and rainfall ranged from 0.00 to 106.9 mm (mean = 4.3 mm).

2.2 | Animal housing and feeding

Eulemur were generally housed in pairs with dependent offspring, while *L. catta*, *P. coquereli*, and *Varecia* were typically housed in

multimale-multifemale groups (Supplementary Material Table S1). Depending on the outdoor temperature at night, animals were kept indoors (room size: approximately 2.13 m length × 2.29 m width × 3.05 m height; one room per adult in the group), given access to attached outdoor chain-link runs (4.27 m length × 2.29 m width × 3.05 m height), housed in outdoor-only chain-link silos (approximately 4.57 m diameter × 5.49 m height for the entire group), or given free-range access to forest enclosures that vary in size from 1.6 to 14.3 acres. For each day and night, we categorized the housing access of subjects into three types: indoor-only, outside access, and forest access. When kept indoors, subjects were exposed to daylight and moonlight via three 30 × 30 cm glass block windows per room. Indoor lighting was set on a timer that automatically tracked the local photoperiod to turn off at astronomical sunset and turn on at astronomical sunrise; our cutoffs for diurnal and nocturnal activity were conservative and based on astronomical twilights (see below).

Animals received food (fresh fruit, vegetables, and monkey chow) twice per day and could access water ad libitum. No food was provided at night, but enclosures may contain provisions from earlier in the day, and outdoor chain-link runs have vines and flowers growing on the mesh. Additionally, all species at the DLC are distributed across each main housing unit; in other words, there are no systematic differences in the species identities of next-door or nearby neighbors. To further control for differences that might arise due to housing conditions, we included random effects in our statistical models (see below).

2.3 | Data collection

To obtain activity data, MotionWatch 8® (CamNtech, Cambridge, United Kingdom) actigraphy data loggers, weighing 7 g, were attached to nylon collars and placed around individuals' necks for 6–8 days. This protocol ensured equal coverage of age and sex across seasons, while seasonal environmental variation was sampled between subjects. Species were monitored across the study period (January–July 2014; Table 2), with specific groups chosen based on availability and as dictated by DLC housing and veterinary schedules. Typically, we collected data on four subjects per week, matching an age/sex pair in a social group of one species to a similar age/sex pair in a social group of another species (e.g., an adult male and female from a *Eulemur* group with an adult male and female from a *P. coquereli* group). Additionally, in most weeks, we collected data from animals in close proximity (but not in adjacent enclosures) to control for ambient noise across the DLC housing units (Supplemental Material Table S2). All animals within a given housing unit can hear disruptive vocalizations such as alarm calls.

2.4 | Data processing

The MotionWatch 8® sensor logs motion data over a user-defined interval, or epoch using a built-in tri-axial accelerometer. The sensor samples data at 50 Hz and accumulates data over the epoch, ultimately assigning it an integer value on a ratio scale. We collected data on the minute, as continuous 1 min sampling is the most commonly used actigraphic method for measuring sleep-wake activity patterns in both human (Ancoli-Israel et al., 2003; Johnson et al., 2007) and non-human

TABLE 1 Study subjects by species and sex

| Taxon | Female | Male | Total |
|--|--------|------|-------|
| <i>Eulemur coronatus</i> | 4 | 5 | 9 |
| <i>Eulemur flavifrons</i> | 2 | 9 | 11 |
| <i>Eulemur mongoz</i> | 6 | 5 | 11 |
| <i>Lemur catta</i> | 14 | 7 | 21 |
| <i>Propithecus coquereli</i> | 10 | 9 | 19 |
| <i>Varecia rubra</i> + <i>V. variegata</i> | 10 | 7 | 17 |
| Total | 46 | 42 | 88 |

TABLE 2 Number of sampled 24-hr cycles by month and species

| Month | <i>Eulemur coronatus</i> | <i>Eulemur flavifrons</i> | <i>Eulemur mongoz</i> | <i>Lemur catta</i> | <i>Propithecus coquereli</i> | <i>Varecia</i> |
|----------|--------------------------|---------------------------|-----------------------|--------------------|------------------------------|----------------|
| January | – | 6 | 12 | 18 | 12 | – |
| February | – | 12 | 6 | 24 | 17 | 12 |
| March | 6 | – | – | 30 | 36 | – |
| April | 26 | 36 | 10 | 16 | 16 | – |
| May | – | – | 38 | 24 | – | 22 |
| June | 25 | 6 | – | – | 15 | 38 |
| July | – | 6 | – | 6 | 6 | 22 |

For complete scheduling by subject, see Supplementary Material Table S2.

primates (Sri Kantha & Suzuki, 2006). Although the MotionWatch 8® also records light, the sensor was often blocked by thick fur or huddling behavior and was therefore unreliable; thus, we did not use the devices' light-level recordings.

To classify each minute epoch as either *active* or *inactive*, we assessed the actual behavior associated with various integer values using video recorded from an AXIS® P3364-LVE Network Camera (Axis Communications, Lund, Sweden) with built-in infrared capacity. Observing the histogram of integer values for all epochs in our dataset, we noted a clear break in the data: 45% of the minute epochs in our dataset had integer values of zero, while none had values of 1–3. In the 55% of all epochs that registered at least some activity, the integer value was 4 or greater. Confirming the behavioral validity of this break, video recordings of epochs with values of 0 clearly showed inactivity, whereas values of 4 or greater displayed wakeful activity. However, the lowest values (4–9) represented gross body movements or scanning behavior. Because cathemeral activity patterns are typically identified by the occurrence of substantive activity (e.g., feeding and travelling) during both the day and night (Donati & Borgognini-Tarli, 2006a; Halle, 2006; Tattersall, 1987), we set the cutoff at 10, which corresponded to epochs with actual physical movement about the enclosure. Epochs were reclassified as either 0 (inactive) or 1 (active), based on whether the raw values were less than 10 or greater than or equal to 10, respectively. Of the minutes that were classified as active, the range in raw values was 10–5073 (Mean = 271 ± SD 330, Median = 163). We acknowledge that operational definitions of cathemeral behavior are challenging; in addition to our behavioral definition, we therefore used widely accepted cathemeral (*Eulemur*) and diurnal (*Propithecus*) taxa as standards for comparison.

To assign minute epochs to “day/diurnal” and “night/nocturnal” periods, we obtained morning and evening astronomical twilight times downloaded from the US Naval Observatory Astronomical Calendar (<http://aa.usno.navy.mil/data>), using geographic coordinates for the DLC. We also downloaded data on lunar illumination from this same source, defined as the proportion of the Moon's area illuminated by direct sunlight to its total area. Used as a proxy for moonlight, these lunar data ranged from 0 (new moon) to 1 (full moon). Group size data were based on the number of individuals in each subject's social group, including infants. Data on age and sex were extracted from DLC records for each animal. Subjects varied in housing conditions (see “Animal housing and feeding”), with some individuals having forest access. We removed nights when subjects had forest access from the

analysis for several reasons. Firstly, these enclosures vary greatly in terms of what the animals experience, both in terms of sleeping substrates and sleep disturbances. Secondly, the animals likely varied in their habituation to these outdoor conditions, thus increasing variability in activity patterns. Lastly, we could not determine whether subjects were actually sleeping in the forest or in their artificial enclosures.

We generated two dependent measures in our analyses. These measures provided complementary estimates of the activity expressed by each subject over each 24-hr cycle. For the first measure, *mean night activity*, we calculated the average number of active minutes at night per hour (i.e., the number of active minutes at night divided by the total number of night hours, which varied by season). Here, higher values represented greater nocturnal activity. For the second measure, *day/night activity ratio*, we divided the *mean day activity* by the *mean night activity*. Here, values equal to 1 indicated the same degree of activity during day and night hours, with values below 1 indicating more activity at night relative to day activity and values above 1 indicating less activity at night relative to day activity. Analyzed together, a subject that had a larger mean night activity and a smaller day/night activity ratio would be interpreted as more cathemeral.

2.5 | Data analysis

In total, we analyzed data on 503 data-logged 24-hr cycles (12,072 hours) across 88 individual lemurs (for sampling coverage across the study period, see Table 2 for data at the species level and Supplementary Material Table S2 for individual data). All analyses were conducted in R (version 3.1.3; R Development Core Team, 2015; <https://www.r-project.org>). To assess the predictors of cathemerality, we ran linear mixed effects models for each of our two dependent variables using the “lme4” package (Bates, Maecher, Bolker, & Walker, 2015), with species as the primary factor of interest. Because of the limited number of subjects for *V. variegata* ($N = 4$), both *Varecia* species were analyzed together ($N = 17$). The reference taxon in our primary models was *P. coquereli*, a diurnal species and an out-group to the Lemuridae. To make statistical comparisons to a cathemeral species, we reran the models with a *Eulemur* species as the reference taxon. In all models, we included sex, age, and group size as covariates, along with lunar illumination (i.e., moonlight), day length (number of hours between astronomical dawn and dusk), and housing access (1 = indoor-only, 2 = outside access; see “Animal housing and

feeding"). An interaction was included between lunar illumination and housing access because we expected that moonlight would be more likely to have a positive effect on nocturnal activity when individuals had outside access. To control for repeated measures, we included "subject" and "group ID" (defined as a unique set of conspecifics) as random effects, and we obtained coefficients based on optimization of the log-likelihood. We used natural log transformations of the dependent variables to normalize the residuals of our models. We used the "MuMIn" package (version 1.15.6, Kamil Bartoń, 2015, <https://cran.r-project.org/web/packages/MuMIn>) to average models based on Akaike information criterion (AIC) (Burnham & Anderson, 2002). We averaged all models with delta AIC less than 10. AIC scores provide a measure of the relative quality of a model for a given dataset; delta AIC scores provide a measure of each model relative to the best fit model.

To interpret our results, we used standardized coefficients and standard errors based on the "full average" (i.e., with shrinkage, as opposed to the conditional average). In models where a parameter is absent, this method sets the corresponding coefficient and variance to zero for the purposes of the average, which provides more conservative estimates and does not bias estimates away from zero. We also examined confidence intervals based on the parameter estimates.

3 | RESULTS

3.1 | Measure 1: Mean night activity

After controlling for all covariates, the three *Eulemur* species were among the most active at night (range: $\beta = 0.25\text{--}0.31$; SE = 0.12–0.14; Table 3), while *P. coquereli* showed the least amount of nocturnal activity (Figures 2 and 3). *Varecia* exhibited more nocturnal activity than *P. coquereli* ($\beta = 0.29 \pm \text{SE } 0.13$), while the nocturnal activity of *L. catta* was between that of *Eulemur* and *P. coquereli* ($\beta = 0.14 \pm \text{SE } 0.11$). Furthermore, the 95% confidence intervals for *Varecia* did not overlap with *P. coquereli*, while the 95% confidence intervals for *L. catta* did overlap (Figure 3). In the version of the model with *E. mongoz* as the reference taxon (lowest mean night activity of all *Eulemur*), *L. catta*

TABLE 3 Effect of species and other predictor variables on mean night activity

| Predictor | Coefficient | s.e. | Z |
|-------------------------------|-------------|-------|-------|
| Species: <i>E. coronatus</i> | 0.331 | 0.137 | 2.424 |
| Species: <i>E. flavifrons</i> | 0.262 | 0.122 | 2.149 |
| Species: <i>E. mongoz</i> | 0.252 | 0.121 | 2.084 |
| Species: <i>L. catta</i> | 0.143 | 0.112 | 1.277 |
| Species: <i>Varecia</i> | 0.291 | 0.131 | 2.223 |
| Age | −0.316 | 0.080 | 3.945 |
| Group size | 0.063 | 0.099 | 0.634 |
| Sex: male | −0.055 | 0.075 | 0.734 |
| Housing: outside access | −0.169 | 0.050 | 3.408 |
| Moonlight | −0.013 | 0.063 | 0.211 |
| Moonlight X housing | −0.001 | 0.050 | 0.019 |
| Day length | 0.032 | 0.074 | 0.437 |

The output of a linear mixed model with *P. coquereli* as the reference category for "species," female as the reference category for "sex," and indoor-only access as the reference category for "housing." Subject and group identity are included as random effects. Positive coefficients indicate increased nocturnal activity compared to the reference taxon, while negative coefficients indicate reduced nocturnal activity.

showed a greater difference ($\beta = -0.17 \pm \text{SE } 0.14$), reflecting less nocturnal activity, than did *Varecia* ($\beta = 0.00 \pm \text{SE } 0.11$).

Apart from the effect of species, older individuals and those with outside access showed reduced levels of nocturnal activity (Table 3). We did not observe any sizable effect of sex, group size, moonlight, or day length.

3.2 | Measure 2: Day/night activity ratio

Based on the ratio of mean day activity to mean night activity, all individuals in our study were more active during the day than at night (middle 80% interval: 1.95–6.35). After controlling for all covariates, *P. coquereli* exhibited the greatest levels of activity during the day relative to the night (Figure 4 and Table 4). The three *Eulemur* species generally showed the lowest ratios of diurnal to nocturnal activity, differing substantially from *P. coquereli* (range: $\beta = -0.21$ to -0.41 ; SE = 0.12–0.14). Compared to *P. coquereli*, *Varecia* activity levels were

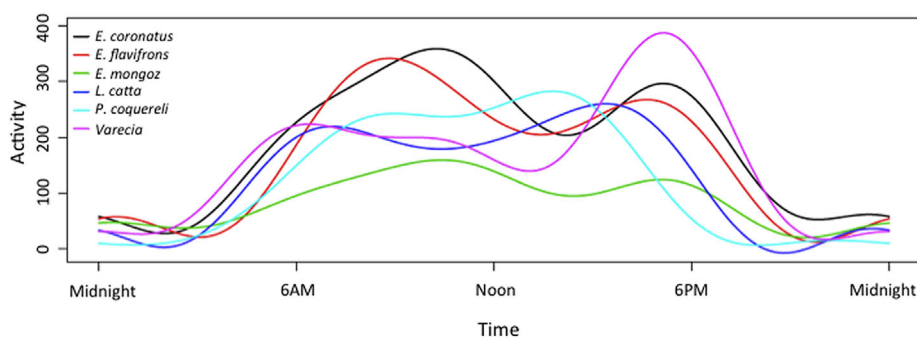


FIGURE 2 Functional linear modeling was used to characterize and illustrate circadian activity. Raw actigraphy data were read into the R statistical software for analysis using the "actigraphy" package (version 1.3.2, William Shannon, Tao Li, Hong Xian, Jia Wang, Elena Deych, and Carlos Gonzalez, 2016, <https://CRAN.R-project.org/package=Actigraphy>). Individuals' data across the 24-hr cycle were averaged and fit by a nine basis Fourier expansion. The mean circadian activity pattern within each taxa is shown by the uniquely colored lines

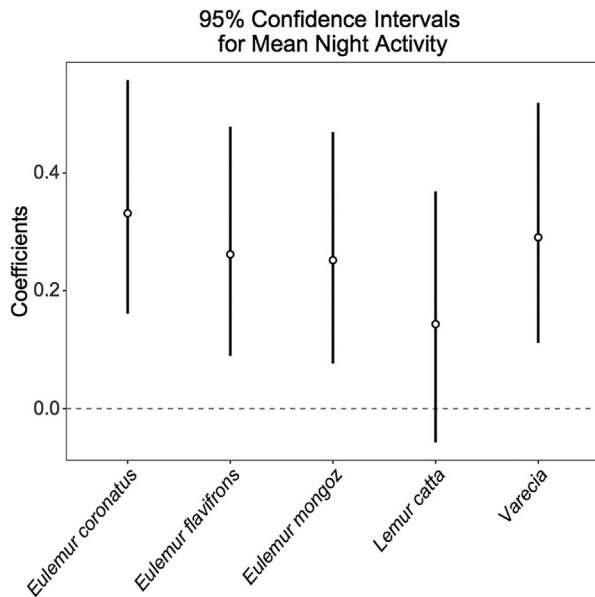


FIGURE 3 Effect sizes and 95% confidence intervals for “species” in the averaged model of mean night activity. Positive coefficients indicate increased nocturnal activity compared to the diurnal *P. coquereli*, the out-group and reference taxon in this analysis

also relatively higher at night than during the day ($\beta = -0.28 \pm \text{SE } 0.13$). Again, *L. catta* had an intermediate activity pattern between the *Eulemur* species and *P. coquereli* ($\beta = -0.17 \pm \text{SE } 0.12$). The 95% confidence intervals for *Varecia* did not overlap with *P. coquereli*, while the 95% confidence intervals for *L. catta* did overlap (Figure 3). In the version of the model with *E. flavifrons* as the reference taxon (highest day/night activity ratio of all *Eulemur*), *L. catta* showed a greater difference

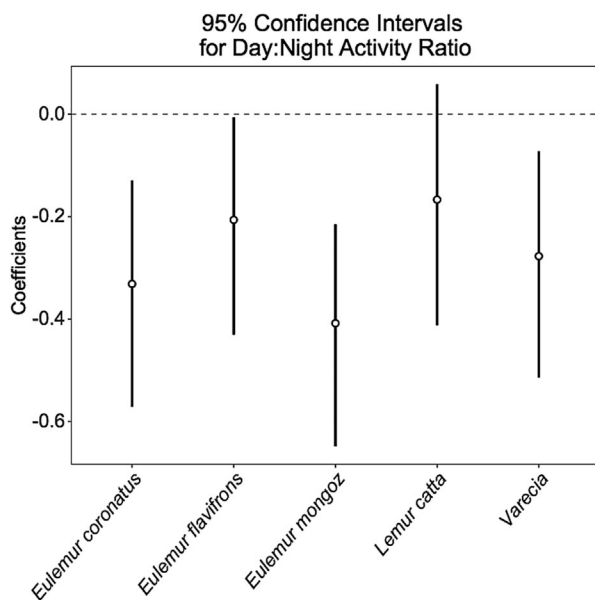


FIGURE 4 Effect sizes and 95% confidence intervals for “species” in the averaged model of day:night activity ratio. Negative coefficients indicate increased nocturnal activity relative to diurnal activity compared to the diurnal *P. coquereli*, the out-group and reference taxon in this analysis

TABLE 4 Effect of species and other predictor variables on day/night activity ratio

| Predictor | Coefficient | s.e. | Z |
|-------------------------------|-------------|-------|-------|
| Species: <i>E. coronatus</i> | -0.331 | 0.136 | 2.443 |
| Species: <i>E. flavifrons</i> | -0.206 | 0.116 | 1.773 |
| Species: <i>E. mongoz</i> | -0.408 | 0.146 | 2.799 |
| Species: <i>L. catta</i> | -0.167 | 0.123 | 1.351 |
| Species: <i>Varecia</i> | -0.277 | 0.128 | 2.159 |
| Age | 0.043 | 0.070 | 0.616 |
| Group size | -0.060 | 0.104 | 0.581 |
| Sex: male | 0.095 | 0.080 | 1.180 |
| Housing: outside access | 0.240 | 0.050 | 4.797 |
| Moonlight | 0.014 | 0.064 | 0.227 |
| Moonlight X housing | -0.001 | 0.049 | 0.012 |
| Day length | -0.009 | 0.055 | 0.168 |

The output of a linear mixed model with *P. coquereli* as the reference category for “species,” *female* as the reference category for “sex,” and *indoor-only access* as the reference category for “housing.” Subject and group identity are included as random effects. Positive coefficients indicate a higher ratio of diurnal to nocturnal activity compared to the reference taxon, while negative coefficients indicate a lower ratio of diurnal to nocturnal activity.

($\beta = 0.09 \pm \text{SE } 0.14$), reflecting greater diurnal activity relative to nocturnal activity, than did *Varecia* ($\beta = -0.04 \pm \text{SE } 0.12$).

Aside from the effect of species, individuals with outside housing access showed increased diurnal activity relative to nocturnal activity (Table 4). There was no sizable effect of sex, group size, moonlight, or day length. In contrast to analyses of mean night activity, age did not have an effect on the ratio of diurnal to nocturnal activity (Table 4).

4 | DISCUSSION

We characterized the activity patterns of seven captive lemur species across the 24-hr cycle to assess similarities and differences in their endogenous circadian rhythms. All subjects in our study were primarily active during the day, even in the case of the three cathemeral *Eulemur* species, which likely reflects their captive living conditions (see also Rea et al., 2014; Traber & Müller, 2006). Indeed, captive conditions should not only strongly mitigate the supposed benefits of cathemeral behavior (Curtis & Rasmussen, 2002; Donati & Borgognini-Tarli, 2006a), but these conditions are also likely to favor diurnal activity as a response to human activity during daylight hours. Therefore, even if variation in activity patterns among species is consistently observed in the wild, we might not expect to see differences between their captive counterparts. Nevertheless, in support of our first hypothesis, we found differences among the species in our study, particularly between species classified as diurnal and cathemeral in the wild. This suggests that these species have distinct endogenous circadian rhythms that influence their activity, even in the absence of any proximate factors hypothesized to have driven the evolution of activity patterns.

Furthermore, in support of the second hypothesis, the three *Eulemur* species exhibited the greatest amount of nocturnal activity and the lowest day/night activity ratios, which we interpret as signals of cathemerality. This is consistent with studies demonstrating cathemeral behavior in this genus in the wild, including all three of the species in the present study (*E. coronatus*: Freed, 1996; Wilson, Stewart, Ramangason, Denning, & Hutchings, 1989; *E. flavifrons*: Schwitzer, Kaumanns, Seitz, & Schwitzer, 2007; *E. mongoz*: Curtis et al., 1999; Rasmussen, 1999; Tattersall & Sussman, 1975). Finally, although we observed some variation among the three *Eulemur* species, our measures of mean night activity and day/night activity ratio do not allow us to draw conclusions regarding variation in the expression of cathemeral behavior in the corresponding wild populations.

By contrast, *P. coquereli* displayed the lowest level of nocturnal activity and the highest day/night activity ratio, supporting previous claims of strict diurnality in this genus (*P. coquereli*: Rea et al., 2014; *P. verreauxi*: Erkert & Kappeler, 2004). As for *L. catta*, we found an intermediate activity pattern between the *Eulemur* species and *P. coquereli*, which is somewhat consistent with recent studies demonstrating cathemeral behavior in wild *L. catta* populations (Donati et al., 2013; LaFleur et al., 2014). Interestingly, based on our two measures of cathemerality, *Varecia* exhibited greater differences in comparison to *P. coquereli* than did *L. catta*. Likewise, *L. catta* was more dissimilar compared to the *Eulemur* species than was *Varecia*.

The finding that *Varecia* are relatively active at night substantiates anecdotal observations reported in reviews of cathemeral behavior (Donati & Borgognini-Tarli, 2006a; Wright, 1999). In addition, it replicates the results of a previous study at the DLC conducted on a smaller number of subjects (Rea et al., 2014), which found that nocturnal activity in *Varecia* was comparable to that of *E. mongoz*. It is also important to consider interspecific variation within the genus *Varecia*. In the present study, however, there were only four *V. variegata* subjects (compared to 13 subjects of *V. rubra*), precluding any meaningful analysis by species. A broader sample across both species is needed to reveal whether these effects are driven by just one species or apply more widely to the entire genus. Furthermore, although the current study presents evidence suggesting that *Varecia* is cathemeral, follow-up studies of wild *Varecia* populations are needed to assess the generality of this pattern and the factors that influence its expression.

If *Varecia* indeed has cathemeral tendencies, why has this activity pattern not been previously demonstrated in the wild, aside from a few anecdotal reports (see Donati & Borgognini-Tarli, 2006a; Wright, 1999)? One possibility is that too few populations have been studied. Only in the past few years has cathemeral behavior been observed in wild studies of *L. catta*, and this has been achieved by studying new populations (Donati et al., 2013; LaFleur et al., 2014). Moreover, cathemeral behavior is by definition associated with extreme flexibility (Halle & Stenseth, 2000), and we know that a species may exhibit both cathemeral and diurnal activity patterns at different times of year. In the case of *V. rubra*, most research on this species has been conducted on a single population in Masoala National Park (Rigamonti, 1993; Vasey, 2000, 2002). Although only diurnality has been reported at this site (Vasey, 2005), annual variation across the 24-hr cycle has yet to be

studied, to our knowledge. Furthermore, this species is restricted to a single peninsula in northeastern Madagascar but may have ranged over a larger geographic area in the past (Mittermeier et al., 2010), in which case cathemerality may simply be absent in the Masoala region. Similarly, to date there has been no published study demonstrating cathemeral activity patterns in *V. variegata*, although it also has not received much attention in the wild (but see Balko & Underwood, 2005; Britt, 2000; Morland, 1991). Given its larger latitudinal range (Mittermeier et al., 2010), the present findings suggest that further study of *V. variegata* may reveal populations that exhibit cathemeral activity patterns.

Examining the covariates in our models, age and housing access had some influence on activity. Younger individuals were more active at night compared to older individuals, but we did not find an effect of age when looking at the ratio of diurnal to nocturnal activity, suggesting that younger individuals are generally more active but not more cathemeral. We also found that individuals expressed a more cathemeral activity pattern when they were restricted to the indoor enclosures. This result is counterintuitive because subjects presumably had fewer environmental stimuli when inside; however, individuals with indoor-only access may also sleep more securely, resulting in more efficient sleep bouts and increased activity during parts of the night (David Samson et al. unpublished manuscript).

We did not find any notable effect among the other environmental covariates. Firstly, day length showed no effect, which is unsurprising given that both of our measures controlled for day length by analyzing hourly activity rates. Studies in the wild that found effects of day length have specifically measured the total amount of activity during the day and night (Curtis et al., 1999; Donati et al., 2009; Kappeler & Erkert, 2003). Secondly, moonlight has been shown to have a central role in modulating nocturnal behavior of lemurs in the wild (Colquhoun, 1998; Curtis et al., 1999; Donati et al., 2013; Eppley et al., 2015). Thus, it was surprising that neither lunar illumination, nor its interaction with housing access, showed any effect on activity. However, we lacked data to control for cloud cover (Curtis et al., 1999; Donati & Borgognini-Tarli, 2006b; Schwitzer et al., 2007), which may have mitigated the effect of moonlight. In addition, given the dearth of feeding and travel opportunities at night, moonlight may have less of an effect on the behavior of captive individuals than on those in the wild (Traber & Müller, 2006). Lastly, although outdoor temperature influences activity patterns in the wild (Donati & Borgognini-Tarli, 2006a), we could not explore its effect in this study because animals were kept inside during cold nights, thus conflating temperature with housing access.

Although there are advantages to studying captive populations, there are limitations of this approach as well. Firstly, irrespective of any endogenous circadian rhythm, all species in this study were more active during the day than at night, as defined by astronomical twilights. This was true even for cathemeral species and mirrors other studies of captive activity patterns (Rea et al., 2014; Traber & Müller, 2006; but see Santini-Palka, 1994). In other words, our approach can only demonstrate a signal of cathemerality, not the full behavioral repertoire that is associated with this activity pattern. Secondly, even if *Varecia* and *Eulemur* exhibit similar activity patterns in captivity, this

does not guarantee that wild populations of *Varecia* will exhibit cathemeral behavior. However, we hope that these results encourage further study of *Varecia* in the wild.

In summary, we provide evidence that activity patterns in lemurs are variable in captivity and likely reflect endogenous circadian rhythms. We also furnish evidence that *Varecia* may be cathemeral, and in fact may exhibit cathemeral behavior to a greater degree than *L. catta*. Our findings, if supported in wild populations, suggest that cathemerality in Malagasy lemurs evolved along the lineage that gave rise to all Lemuridae, a clade that split from other lemurs between 22.3 and 16.8 mya (Markolf & Kappeler, 2013; see also Finstermeier et al., 2013). This extends further back in time estimates based on the inclusion of *L. catta* among the set of cathemeral lemur species (Donati et al., 2013) and lends support to the hypothesis that cathemerality arose early in lemurid evolution (Tattersall, 1982, 2008).

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