

# Evolutionary Disequilibrium and Activity Period in Primates: A Bayesian Phylogenetic Approach

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**ABSTRACT** Activity period plays a central role in studies of primate origins and adaptations, yet fundamental questions remain concerning the evolutionary history of primate activity period. Lemurs are of particular interest because they display marked variation in activity period, with some species exhibiting completely nocturnal or diurnal lifestyles, and others distributing activity throughout the 24-h cycle (i.e., cathemerality). Some lines of evidence suggest that cathemerality in lemurs is a recent and transient evolutionary state (i.e., the evolutionary disequilibrium hypothesis), while other studies indicate that cathemerality is a stable evolutionary strategy with a more ancient history. Debate also surrounds activity period in early primate evolution, with some recent studies casting doubt on the traditional hypothesis that basal primates were nocturnal. Here, we used Bayesian phylogenetic

methods to reconstruct activity period at key points in primate evolution. Counter to the evolutionary disequilibrium hypothesis, the most recent common ancestor of *Eulemur* was reconstructed as cathemeral at ~9–13 million years ago, indicating that cathemerality in lemurs is a stable evolutionary strategy. We found strong evidence favoring a nocturnal ancestor for all primates, strepsirrhines and lemurs, which adds to previous findings based on parsimony by providing quantitative support for these reconstructions. Reconstructions for the haplorrhine ancestor were more equivocal, but diurnality was favored for simian primates. We discuss the implications of our models for the evolutionary disequilibrium hypothesis, and we identify avenues for future research that would provide new insights into the evolution of cathemerality in lemurs. *Am J Phys Anthropol* 147:409–416, 2012. ©2012 Wiley Periodicals, Inc.

Activity period plays a central role in studies of primate origins and adaptation (Heesy and Ross, 2001), yet fundamental questions remain concerning the evolutionary history of activity period in primates (van Schaik and Kappeler, 1996; Tan et al., 2005; Donati and Borgognini-Tarli, 2006a). The vast majority of haplorrhine primates show diurnal activity patterns (with *Aotus* and *Tarsier* being the only exceptions), while all non-Malagasy strepsirrhines exhibit strictly nocturnal activity patterns. In striking contrast, the lemurs of Madagascar display wide variation in activity period, with some species nocturnal, some diurnal, and others showing flexible patterns of activity at both night and day (i.e., cathemeral activity; Tattersall, 1982). Interestingly, diurnal lemurs show a lack of convergence with diurnal anthropoids in traits related to activity period (e.g., retinal morphology, Kirk, 2004). More specifically, diurnal anthropoids possess a suite of adaptations for diurnal activity, while diurnal lemurs display a mixture of adaptations to day and night activity (van Schaik and Kappeler, 1996).

Primatologists and evolutionary biologists have long attempted to understand the factors that underlie the diverse activity periods and widespread cathemerality of lemurs (van Schaik and Kappeler, 1996; Wright, 1999; Donati and Borgognini-Tarli, 2006a). Four hypotheses have proposed that cathemeral behavior is an adaptation. Under the thermoregulatory hypothesis, cathemeral behavior is a strategy to avoid high daytime temperatures or to offset lower night-time temperatures with increased night-time activity (Tattersall, 1976; Curtis et al., 1999). The food availability/quality hypothesis argues that cathemerality allows lemur species without digestive specializations to maximize the time for which food is being processed, particularly when the proportion

of fibrous food in the diet increases during periods of low food availability or quality (Engqvist and Richard, 1991). The predation hypothesis suggests that flexible activity patterns evolved as an antipredator strategy such that the amount of daytime activity is positively associated with seasonal variation in leaf cover (Curtis and Rasmussen, 2002, 2006). A variant of the predation hypothesis argues that cathemerality represents “temporal crypticity” to avoid predation by the cathemeral fossa (*Cryptoprocta ferox*, Colquhoun, 2006). Finally, the feeding competition hypothesis proposes that cathemerality reduces interspecific feeding competition among sympatric diurnal and nocturnal species by creating a third temporal niche (Tattersall and Sussman, 1990; Curtis and Rasmussen, 2006). These hypotheses are not mutually exclusive, such that any combination of these proposed benefits may have contributed to the evolution of activity period in lemurs. A consensus on the function of cathemerality has failed to emerge, however, as there is

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evidence both for and against each of these hypotheses (Table 1; reviewed in Donati and Borgognini-Tarli, 2006a).

In contrast to the adaptive benefits of these hypotheses, the “evolutionary disequilibrium hypothesis” (EDH) considers a nonadaptive scenario for the evolution of diverse activity periods in lemurs, particularly in the context of cathemeral activity. Evolutionary disequilibrium refers to the “mismatch” between an organism’s biology and environment that can occur when organisms have had insufficient time to adapt to a rapidly changing environment. This concept has been used to explain variation in communities subject to transient climatic conditions, including some coral reefs (Potts, 1983) and deciduous forests (Lechowicz, 1984). Van Schaik and Kappeler (1996) proposed that cathemerality and the presence of nocturnal characteristics in diurnal lemurs represent an example of evolutionary disequilibrium, arguing that human-caused extinctions of large diurnal lemurs and/or diurnal raptors in the past 2,000 years provided an opportunity for nocturnal lemurs to transition into diurnal niches. Thus, although many lemurs increasingly exploit a diurnal lifestyle, they have had insufficient time to evolve the full suite of adaptations to diurnality that is observed in most anthropoid primates. This hypothesis has generated much critical discussion among primate behavioral ecologists (e.g., Wright, 1999; Donati and Borgognini-Tarli, 2006a). Unfortunately, clear tests of the EDH have been elusive. This lack of definitive evidence arises largely because the EDH predicts variation in the degree to which different lemur species show adaptations to diurnality, but it fails to make clear predictions concerning which traits or species should be in lesser or greater “disequilibrium” relative to their nocturnal ancestors. Thus, it is difficult to use the presence or absence of adaptations to test EDH (Kirk, 2004).

However, EDH does make two predictions that can be tested using phylogenetic reconstructions:

**Prediction 1.** Ancestors to extant lemurs should not be reconstructed as having cathemeral activity period (i.e., all ancestral nodes on the lemur phylogeny should be reconstructed as either nocturnal or diurnal), reflecting recent origins of cathemeral behavior.

**Prediction 2.** Recent nodes on lineages leading to cathemeral lemurs should be reconstructed as nocturnal, reflecting the EDH assumption that disequilibrium is caused by rapid shifts of nocturnal lemurs into diurnal niches.

These predictions arise because EDH posits that niche expansions started within the past 2,000 years, well after the major divergence events in lemur evolutionary history. In the present study, we aim to test EDH by assessing statistical support for ancestral states at nodes for which EDH makes specific predictions. In addition to reconstructing ancestral states, we also investigated a model in which we fixed branches leading to cathemeral lemurs and *Lemur catta* as having nocturnal, cathemeral, or diurnal character states 2,000 years ago, with EDH predicting that the model with nocturnal ancestral states is favored relative to the other models. Although *Lemur catta* is typically considered diurnal, recent evidence suggests the possibility of cathemeral behavior (Parga, 2011), and this species has specifically been cited as an example of evolutionary disequilibrium due to pelage, visual adaptations and social behavior (Kappeler and Erkert, 2003).

Previous phylogenetic studies have used parsimony methods to reconstruct activity period in lemurs. However, as Kappeler and Erkert (2003, p 366) point out, “. . . all parsimonious reconstructions of activity patterns among the Lemuriformes revealed that the ancestral activity pattern of the Lemuridae cannot be unequivocally reconstructed with the available activity data.” Indeed, parsimony reconstructions of activity period and related characteristics have produced equivocal results within Lemuridae (Ross, 2000; Heesy and Ross, 2001; however, see Donati and Borgognini-Tarli, 2006a), and thus do not lend support for or against our phylogenetic predictions. In addition, it is now clear that methods based on parsimony suffer from a number of fundamental weaknesses. First, standard parsimony implementations assume equal rates of “gains” and “losses” for a character, yet this assumption is often violated and parsimony methods provide few diagnostic approaches for setting asymmetric rates as a “cost matrix” when reconstructing ancestral states (Omland, 1997; Cunningham et al., 1998; Nunn, 2011). Second, parsimony methods fail to take into account branch lengths—which represent the time since lineages last shared a common ancestor—when reconstructing ancestral states. By ignoring branch lengths, parsimony approaches assume that transitions are equally likely on any branch of the tree. It is more reasonable to assume, however, that evolutionary change is more likely to occur when more time is available for change to take place (Pagel, 1994; Schluter et al., 1997). A final concern is that parsimony does not provide quantitative measures of support for reconstructed states, but rather produces “equivocal” or “unequivocal” results for a given state (Cunningham, 1999; but see Maddison, 1995).

On the basis of these concerns, we applied Bayesian methods to test predictions of the EDH and to investigate the evolutionary history of activity period in primates more generally. Bayesian approaches overcome many of the weaknesses of parsimony by incorporating branch lengths and estimating transition rates between character states. Bayesian reconstructions use an iterative process called Markov Chain Monte Carlo (MCMC) to sample parameters of the underlying evolutionary model in proportion to their probabilities, and also to sample ancestral states at predefined nodes on the tree (Pagel et al., 2004; Pagel and Meade, 2006). Thus, rather than obtaining point estimate reconstructions—as is typical of parsimony methods—Bayesian approaches provide a distribution of ancestral character states and transition rate parameters for the evolutionary model. Bayesian methods also provide a way to run analyses on a sample of trees obtained from a Bayesian phylogenetic analysis, which enables the user to account for uncertainty in the phylogenetic relationships of the species under study (Pagel and Lutzoni, 2002; Pagel et al., 2004; Carnes et al., 2011; Nunn et al., 2011). Finally, it is possible to statistically compare different evolutionary scenarios (Pagel and Meade, 2006), such as scenarios in which particular nodes are fixed to different character states (Pagel and Meade, 2007). Thus, Bayesian methods provide significant advantages over parsimony and are relatively easy to implement (Ronquist, 2004; Nunn, 2011).

In addition to testing the two phylogenetic predictions of EDH, we reconstructed nodes representing the ancestral primate, strepsirrhine, haplorrhine, and anthropoid. Most lines of evidence suggest that basal primates were nocturnal. For instance, the parsimony reconstructions of Heesy and Ross (2001) indicate that opsin genes, chromacy

TABLE 1. Summary of adaptive hypotheses for cathemerality in lemurs

	Adaptive hypothesis	Supporting evidence	Conflicting evidence
Thermoregulation	Increased nighttime activity avoids daytime heat or offsets cold nighttime temperatures (Tattersall, 1976; Curtis et al., 1999)	<ul style="list-style-type: none"> <li>• Cathemeral <i>Eulemur</i> have low metabolic rates and display thermoregulatory behaviors, indicating they face thermoregulatory pressure (reviewed in Donati and Borgognini-Tarli, 2006a)</li> <li>• <i>Eulemur mongoz</i> populations in warm regions show more night activity than populations in cool regions (Tattersall, 1976; 1978)</li> <li>• <i>Hapalemur alaotrensis</i> respond to high daytime temperatures with increased nighttime activity (Mutschler, 1999; 2002)</li> <li>• Inverse relationship was found between night temperature and night activity in several <i>Eulemur</i> species (Curtis et al., 1999; Donati et al., 1999; Kappeler and Erkert, 2003; Overdorff and Rasmussen, 1995)</li> </ul>	<ul style="list-style-type: none"> <li>• Many cathemeral lemurs live in less seasonal habitats and show fluctuations in activity period that are not associated with temperature (Andrews and Birkinshaw, 1998; Donati and Borgognini-Tarli, 2006a; Tarnaud, 2006)</li> </ul>
Food availability and quality	Distributing activity across 24-h cycle maximizes food processing time for dietary nonspecialists during lean periods (Engqvist and Richard, 1991)	<ul style="list-style-type: none"> <li>• Lemurs endure dramatic seasonal fluctuations in food quality/availability (Wright, 1997)</li> <li>• Cathemeral <i>Eulemur</i> species cannot retain food in the gut for extended periods, so compensating by extending food processing time may be necessary (Overdorff and Rasmussen, 1995)</li> <li>• Some field studies show increased cathemerality in <i>Eulemur</i> species during lean periods (Tattersall, 1979; Overdorff, 1988; Colquhoun, 1993)</li> </ul>	<ul style="list-style-type: none"> <li>• Some <i>Hapalemur</i> are also cathemeral, but <i>Hapalemur</i> are specialized folivores with gut passage rates 25 times slower than <i>Eulemur</i> (Overdorff and Rasmussen, 1995)</li> <li>• The diminished ability of <i>Eulemur</i> species to process fibrous food has been questioned (Kay and Hylander, 1978; Sheine, 1979)</li> <li>• Many field studies find no relation between diet and cathemerality in <i>Eulemur</i> (reviewed in Donati and Borgognini-Tarli, 2006a)</li> </ul>
Predation	Cathemerality allows responses to seasonal changes in predation risk from diurnal raptors due to seasonal variation in leaf cover (reviewed in Curtis and Rasmussen, 2002), or may represent “temporal crypticity” to avoid predation from cathemeral fossa (Colquhoun, 2006)	<ul style="list-style-type: none"> <li>• Lemurs face significant predation pressure from both diurnal raptors and fossa (reviewed in Colquhoun, 2006)</li> <li>• <i>Eulemur</i> species possess specific alarm calls for different predatory birds (Fitchell and Kappeler, 2002)</li> <li>• Some field studies show seasonal variation in lemur activity period associated with changes in leaf cover (reviewed in Donati and Borgognini, 2006a)</li> </ul>	<ul style="list-style-type: none"> <li>• Some field studies report seasonal cathemerality in lemurs with year-round leaf cover (Donati and Borgognini-Tarli, 2006b), indicating cathemerality is not in response to variation in exposure to diurnal raptors</li> <li>• Activity peaks for cathemeral lemurs fall at dusk and dawn, providing predictable opportunities for predators, casting doubt on the hypothesis that cathemerality is “temporal crypticity” to avoid fossa predation (reviewed in Donati and Borgognini-Tarli, 2006a)</li> </ul>
Interspecific feeding competition	Cathemerality reduces interspecific feeding competition among diurnal and nocturnal species by creating a distinct temporal niche (Curtis and Rasmussen, 2006)	<ul style="list-style-type: none"> <li>• Some sympatric <i>Hapalemur</i> and <i>Eulemur</i> species show nonoverlapping cathemeral activity periods in the wild (Tan, 1999; Rasmussen, 1999)</li> <li>• <i>Hapalemur simus</i> and <i>Hapalemur griseus</i> show different profiles of cathemerality together in captivity (Santini-Palka, 1994)</li> </ul>	<ul style="list-style-type: none"> <li>• Many cathemeral lemurs face neither strong interspecific feeding competition nor occur in sympatry with congeneric species (Donati and Borgognini-Tarli, 2006b; Tarnaud, 2006; Mutschler, 1999; 2002)</li> </ul>



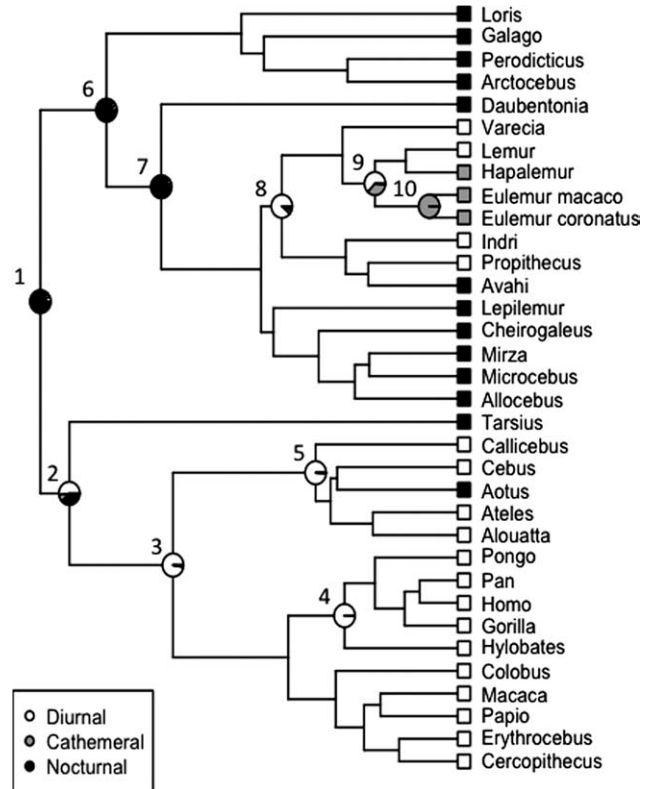
type, and activity pattern in extant primates are consistent with nocturnal ancestry for primates, strepsirrhines, and haplorrhines. Further support comes from living and fossil primates (Kay and Ross, 1997). However, some studies have suggested that ancestral primates were diurnal or cathemeral. For example, Ni et al. (2004) proposed that a 55 million-year-old primate skull showed evidence for diurnality, while others used genetic evidence from opsin genes to argue that basal primates were diurnal or possibly cathemeral (Tan and Li, 1999; Tan et al., 2005; however, see Zhao et al., 2009). Thus, a secondary goal of our study is to provide the first Bayesian reconstructions of activity period at key nodes in primate evolution. To assess the performance of the methods for this goal, we also investigated activity period in several “validation” nodes where activity period is relatively certain based on fossil and other evidence.

## METHODS

We downloaded 100 dated trees comprising 230 primate species from Version 2 of the *10kTrees* website (Arnold et al., 2010). Data on activity period were obtained from Rowe (1996) with additional sources for ambiguous species (see Supporting Information Table S1). Activity period can be recorded as a three-state character (nocturnal, cathemeral, and diurnal states), or as a two-state character (nocturnal and diurnal states, with cathemeral species coded as polymorphic). For this study, we coded the data both ways and repeated all analyses under both coding schemes (Supporting Information Table S1).

We tested EDH by reconstructing ancestral states of four clades containing cathemeral lemurs: *Eulemur*, *Eulemur/Hapalemur/Lemur*, Lemuridae/Indriidae, and Lemuroidea (Fig. 1). We also reconstructed the ancestral primate, strepsirrhine, and haplorrhine under the general expectation that they would be nocturnal, and the ancestral state for anthropoids with the prediction that diurnality is primitive. To assess the performance of the methods, we reconstructed ancestral nodes for two additional clades for which activity period is well established: platyrrhines and hominoids, both of which are thought to be diurnal based on comparative analyses of extant species and fossil evidence (Rasmussen and Simons, 1992; Kay and Kirk, 2000; Heesy and Ross, 2001).

Inference of ancestral states requires an evolutionary model describing transitions among character states, but rarely does one know in advance which model of evolution to use. We therefore implemented reversible jump MCMC to select evolutionary models in a Bayesian inference framework, with the frequency of different models sampled in proportion to their effect on the likelihood of the data (Pagel and Meade, 2006). Models that make the data more likely are selected more frequently, with the frequency of each model reflecting its posterior probability. We performed all analyses using the MultiState option in the program BayesTraits (Pagel et al., 2004; Pagel and Meade, 2007). For each analysis we ran independent MCMC chains for 10,000,000 iterations, sampling parameter values every 100 iterations and discarding the first 300,000 iterations as “burnin,” which is the initial part of the run when the MCMC chain is climbing the likelihood surface, and thus prior to sampling from the posterior probability distribution of parameters. Three independent chains were run to ensure convergence of parameter estimates to the same



**Fig. 1.** Reconstructions of activity period at ten nodes in the primate phylogeny, with activity period coded as a three-state character. Pie charts represent the posterior probability of each state at each node from the unconstrained reversible jump model. For simplicity, we display only genus names corresponding to a subset of the 230 species included in our analysis, such that important variation in activity patterns is shown (with the exception of *Eulemur*). For *Eulemur*, two species are shown to provide a node on the figure for the ancestor of all members of this genus. See Supporting Information Table S1 for a full list of species in the analysis. Reconstructed nodes: 1) Primates; 2) haplorrhines; 3) anthropoids; 4) hominoids; 5) platyrrhines; 6) strepsirrhines; 7) Lemuroidea; 8) Lemuridae/Indriidae; 9) *Eulemur/Hapalemur/Lemur*; 10) *Eulemur*.

posterior distribution. We used an exponential prior for model parameters, drawing the mean of the exponential prior from a uniform “hyperprior,” which is simply a prior distribution for the parameter (in this case, the mean of an exponential distribution) defining the prior distribution for the transition rate parameters, set from 0 to 10. To assess whether the prior had any effects on the outcome, we also ran analyses using a uniform prior with no hyperprior and examined maximum likelihood reconstructions in BayesTraits. These analyses produced qualitatively similar results.

We assessed statistical support for reconstructions with two techniques. First, nodes were “fossilized” at each character state (Pagel, 1994). Node fossilization involved setting the node of interest to a given state and calculating the harmonic mean of the log likelihoods for the MCMC chain under that fossilization. This process was repeated for different states and a Bayes factor was used to compare the models, where the Bayes factor is twice the difference in the harmonic means (Kass and Raftery, 1995; Pagel and Meade, 2006). Second, we used an unconstrained Bayesian model based on reversible jump MCMC to incorporate uncertainty in which param-

TABLE 2. Support for alternative character states at nodes

Clade	Harmonic mean: three-state coding			Bayes factor	Harmonic mean: two-state coding		Bayes factor
	Diurnal	Cathemeral	Nocturnal		Diurnal	Nocturnal	
Primates	-49.91	-49.88	<b>-46.77</b>	6.22 <sup>b</sup>	-26.36	<b>-24.66</b>	3.4 <sup>a</sup>
Strepsirrhines	-51.45	-50.87	<b>-47.16</b>	7.42 <sup>b</sup>	-27.6	<b>-24.91</b>	5.38 <sup>b</sup>
Lemuroidea	-49.91	-50.5	<b>-47.33</b>	5.16 <sup>b</sup>	-27.85	<b>-24.74</b>	6.22 <sup>b</sup>
Lemuridae/Indriidae	<b>-47.88</b>	-50.36	-48.63	1.50	<b>-25.4</b>	-26.12	1.44
<i>Hap/Eu/Lemur</i>	<b>-47.71</b>	-50.92	-52.67	6.42 <sup>b</sup>	<b>-24.91</b>	-29.3	8.87 <sup>b</sup>
<i>Eulemur</i>	-54.37	<b>-47.76</b>	-59.97	13.22 <sup>c</sup>	<b>-25.38</b>	-28.81	6.86 <sup>b</sup>
Haplorrhines	-49.85	-50.1	<b>-46.9</b>	5.90 <sup>b</sup>	-26.2	<b>-24.94</b>	2.52 <sup>a</sup>
Anthropoids	<b>-45.07</b>	-51.71	-49.43	8.72 <sup>b</sup>	<b>-22.73</b>	-27.29	9.12 <sup>b</sup>
Platyrrhines	<b>-47.33</b>	-52.51	-54.39	10.36 <sup>c</sup>	<b>-24.34</b>	-29.48	10.28 <sup>c</sup>
Hominoids	<b>-47.51</b>	-55.54	-56.63	16.06 <sup>c</sup>	<b>-24.82</b>	-34.05	18.46 <sup>c</sup>

Note: Results are based on fixing ("fossilizing") nodes to particular character state for the common ancestor of each clade shown in the table. Bold indicates best-supported model for each coding regime based on the Bayes factor. In the three-state coding, the Bayes factor test compares the best two models.

<sup>a</sup> Positive evidence (>2).

<sup>b</sup> Strong evidence (>5).

<sup>c</sup> Very strong evidence (>10).

TABLE 3. Posterior probabilities of each state from the unconstrained reversible jump analysis

Clade	Posterior probabilities: three-state coding			Posterior probabilities: two-state coding	
	Diurnal	Cathemeral	Nocturnal	Diurnal	Nocturnal
Primates	0.07	0.06	<b>0.87</b>	0.14	<b>0.86</b>
Strepsirrhines	0.07	0.06	<b>0.87</b>	0.053	<b>0.947</b>
Lemuroidea	0.05	0.06	<b>0.89</b>	0.11	<b>0.89</b>
Lemuridae/Indriidae	<b>0.89</b>	0.06	0.05	<b>0.98</b>	0.02
<i>Hap/Eu/Lemur</i>	<b>0.63</b>	0.36	0.01	<b>0.98</b>	0.02
<i>Eulemur</i>	0.0001	<b>0.9998</b>	0.0001	0.5	0.5
Haplorrhines	<b>0.52</b>	0.1	0.38	<b>0.71</b>	0.29
Anthropoids	<b>0.97</b>	0.02	0.01	<b>0.98</b>	0.02
Platyrrhines	<b>0.992</b>	0.007	0.001	<b>0.98</b>	0.02
Hominoids	<b>0.994</b>	0.005	0.001	<b>0.999</b>	0.001

Note: Bold indicates the ancestral state with the strongest support.

eters to include in the model and whether different parameters should be assigned the same (symmetrical) rate or different (asymmetrical) rates. In this framework, the MCMC chain generates a posterior distribution of models, parameter estimates, and ancestral state reconstructions at each node. For a given node, the "posterior probability" of each ancestral state at the node is the probability of that state averaged over the posterior distribution; it provides a measure of the plausibility of different evolutionary scenarios given our data and model.

To assess statistical support for a model in which cathemeral lemurs and *Lemur catta* are forced to have nocturnal ancestry 2,000 years ago, nodes were added 2,000 years from the present on each of these lineages and fossilized as either nocturnal, cathemeral or diurnal. These models were then compared using Bayes factors.

## RESULTS

Results were similar for two and three-state coding schemes using the fossilization approach (Table 2). Under both coding schemes, we found positive evidence for one state over the others at every node except for Lemuridae/Indriidae. These analyses supported reconstructions of nocturnality for the ancestors of primates, strepsirrhines, haplorrhines, and lemurs. Diurnality

was favored for *Eulemur/Hapalemur/Lemur*, and as expected, for our validation nodes (platyrrhines and hominoids). Three-state coding strongly favored cathemerality for the ancestor of *Eulemur*, while two-state coding favored diurnality. Thus, counter to Prediction 1, we found no support for an absence of cathemeral activity among nodes in the lemur adaptive radiation, and counter to Prediction 2, extant cathemeral lineages showed evidence for diurnal ancestry.

The unconstrained Bayesian model produced qualitatively similar results to node fossilization (Table 3). Under both two- and three-state coding, greater than 85% support was found for nocturnality in the ancestral primate, strepsirrhine, and Lemuriform. However, our reconstruction for haplorrhines was more ambiguous, with 71% of models favoring diurnality under two-state coding and only 52% of models favoring diurnality under three-state coding. The Lemuridae/Indriidae node was reconstructed as diurnal in over 85% of models under both coding schemes, while the *Eulemur/Hapalemur/Lemur* node was reconstructed as diurnal in 98% of models under two-state coding, but only 63% support under three-state coding (with over 30% support for cathemerality). Our strongest result was for the ancestor of *Eulemur*, which was reconstructed as cathemeral in over 99% of models with three-state coding, and two-state coding yielded percentages of 50% diurnal and 50% nocturnal (Table 3). We interpret

the latter result as possible evidence for cathemerality because cathemeral primates are coded as diurnal/nocturnal (polymorphic) in the two-state coding scheme, so reconstructions that are 50% diurnal and 50% nocturnal can be interpreted either as uncertainty or as cathemerality (the conflation of uncertainty and cathemerality disappears in three-state coding). We found greater than 97% support for diurnality in the ancestral anthropoid and in our test nodes, platyrrhines and hominoids.

Finally, we compared three models in which nodes were added to cathemeral lineages and *Lemur catta* at 2,000 years from present and fossilized as nocturnal, cathemeral or diurnal. In this scenario, EDH predicts nocturnal activity patterns. However, comparison of Bayes factors yielded extremely strong support for cathemeral ancestry over either diurnal ancestry (Bayes factor = 20) or nocturnal ancestry (Bayes factor = 30). Indeed, diurnal ancestry received more support than nocturnal ancestry (Bayes factor = 10), indicating that recent nocturnal ancestry is the least likely scenario for these lineages.

## DISCUSSION

By using recent phylogenetic data and Bayesian methods to reconstruct activity period at key points in primate evolution, our study provides insights into the evolution of activity period in lemurs and early primates. Contrary to the EDH prediction that cathemerality has a very recent origin, our models strongly supported the conclusion that cathemerality was present in lemurs in the last common ancestor of *Eulemur*. The age of this node is well before the hypothesized transition to diurnality under the EDH, with estimated dates of 12.34 ( $\pm 0.26$ ) mya in the trees downloaded from Arnold et al. (2010) and 9.7 mya (95% credible interval of 6.5–13.7 mya) in Yoder and Yang (2004). The strength of statistical support for this reconstruction was similar to our reconstructions for platyrrhines and hominoids, where other evidence strongly indicates a diurnal activity pattern (Rasmussen and Simons, 1992; Kay and Kirk, 2000; Heesy and Ross, 2001). Further, our results failed to support the phylogenetic prediction of EDH that cathemeral lemurs evolved from nocturnal ancestors.

Evidence against the EDH suggests that the one or more of the adaptive hypotheses play an important role in the evolution of cathemerality, including hypotheses related to thermoregulation, food quality and availability, predation risk, and feeding competition. As shown in Table 1, field studies have found evidence both for and against these adaptive hypotheses. However, even if a unitary adaptive explanation for cathemerality is not possible, the question we should be asking based on our findings is still an adaptive one: what factors drove a diurnal lineage (or two, if we assume independent origins of cathemerality in *Eulemur* and *Hapalemur*) to evolve cathemerality in the late Miocene? It seems plausible that multiple forces influenced the evolution of activity period in lemurs, and further studies examining the ecological and physiological correlates of cathemerality in primates and other mammals should shed further light on the adaptive basis of cathemerality (Donati and Borgognini-Tarli, 2006).

Despite strong statistical support for an early origin of cathemerality, an alternative explanation for the observed pattern is compatible with EDH. Specifically, it could be that many closely related species are cathem-

eral because they shared characteristics or environments that exposed them to similar selection pressures or made it easier for them to transition into diurnal niches. In other words, the transitions could be occurring convergently in closely related lineages, which would make it appear as if cathemerality was present in the ancestor of these species and would be incorrectly interpreted as evidence against EDH. However, this “extreme convergence” scenario is unlikely given the strength and specificity of selection for diurnal activity that would have had to occur in *Eulemur*, *Lemur*, and some *Hapalemur* species, but not in other lemur species. Convergent evolution in so many closely related species implies that these lineages must have shared some critical physiological or ecological factors that predisposed them to this rapid evolution, but these genera exhibit wide variation in their ecology (*Eulemur* species in particular occupy diverse habitats across a wide geographic range in Madagascar; Ossi and Kamilar, 2006). These cathemeral lineages also share many aspects of their physiology and environment with lemurs that are not thought to be in disequilibrium (Wright, 1999). For these reasons we argue that the convergent evolution scenario, and thus the EDH scenario, is unlikely. However, by focusing on activity period rather than specific adaptations to diurnal activity, our analyses do not directly test the hypothesis that adaptations to diurnality are convergent in extant lemurs (i.e., EDH). Below, we highlight future studies that could provide more definitive tests of whether adaptations to diurnal activity evolved independently in cathemeral taxa and *Lemur catta*.

As mentioned in the Introduction, it is difficult to test EDH in a comparative context because it is unclear whether the presence or absence of particular adaptations to diurnality supports or contradicts EDH. For example, cathemeral lemurs are intermediate between nocturnal and diurnal lemurs in aspects of visual morphology, and this has been used to argue that these lemurs are well adapted to cathemeral life (Kirk, 2004). The problem is that EDH also predicts that lemurs in disequilibrium will possess intermediate features, as well as adaptations to diurnality and vestiges of their nocturnal past. Thus, the nocturnal-like circadian rhythms of *Eulemur* (Erkert, 1989) and the nocturnal-like pelage of *Lemur catta* (van Schaik and Kappeler, 1996) can be used in conjunction with visual adaptations to diurnality (Kirk, 2004) to argue that these lemurs are in disequilibrium. In light of these issues, we suggest that future tests of EDH should not rely on morphological or physiological comparisons. Molecular data could help detect recent selection at the population level and distinguish between convergent and homologous adaptations. As EDH proposes that selection pressure for diurnal adaptations has been present for only 2,000 years, the expectation is that adaptations will occur primarily through shifts in the frequencies of existing alleles rather than through rare mutations. If low frequency alleles related to activity pattern have risen rapidly in frequency in the past 2,000 years, then we expect to find linkage disequilibrium at these loci. On the other hand, if comparative genetic studies identify adaptations to daytime activity that are conserved among cathemeral and diurnal lemurs at the molecular level, this would strongly suggest that daytime activity has a long evolutionary history. Genes related to circadian rhythms (Shimomura et al., 2001) or photoreception (Yokoyama, 1997) would be strong candidates for such studies. A second



prediction is that adaptations to diurnality should correlate with genetic diversity across species and populations. Specifically, species with larger effective population sizes are expected to be more adapted to diurnality, since standing genetic variation should be greater in larger effective populations (see also van Schaik and Kappeler, 1996).

In the present study, we also reconstructed nodes of basal primates with the expectation that nocturnality is primitive for primates, strepsirrhines, and haplorrhines, while diurnality evolved first at the base of the anthropoid clade (Heesy and Ross, 2001). With one minor exception, our reconstructions for early primates are qualitatively similar to those of Heesey and Ross (2001), who conducted a similar analysis in a parsimony framework. The ancestral primate and strepsirrhine were unambiguously reconstructed as nocturnal, while the ancestral anthropoid was reconstructed as diurnal. Surprisingly, our reconstruction for haplorrhines was equivocal. While Heesy and Ross (2001) found strong evidence that the ancestral haplorrhine was diurnal, our results are much less certain, indicating that the phylogenetic reconstruction of activity pattern at this node may be sensitive to the incorporation of branch lengths, the evolutionary model of character transitions, or phylogenetic uncertainty. The ancestral haplorrhine is expected to be diurnal because the nocturnal features of tarsiers appear to be derived (Ross, 2000). Such evidence is not accounted for in our phylogenetic models and must be considered when interpreting results. As our phylogenetic model incorporates branch length information, multiple transitions in activity period are more likely on the long branch leading to the tarsiers. This may contribute to the low statistical support we found for reconstructions at this node. In light of evidence that nocturnality is derived in tarsiers (Ross, 2000), we conclude that diurnality is the most plausible condition for the ancestral haplorrhine.

In summary, we used Bayesian phylogenetic methods to reconstruct the activity pattern at key points in primate evolution. We conclude that early primates were most likely nocturnal, and that diurnality and cathemerality have an ancient history in the lemurs and thus do not represent a state of evolutionary disequilibrium. Our study demonstrates that phylogenetic approaches complement behavioral and ecological studies, such that we are better able to understand which aspects of the environment are (and are not) acting to modify the behaviors of extant species. Such insights can help focus our conservation efforts on forms of ecological change that are most disruptive to the biology of threatened species.

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