

Electronic Supplementary Materials  
The ecology of spatial memory in four lemur species

**Subjects and study participation**

<b>Lemur</b>	<b>Sex</b>	<b>DOB</b>	<b>Studies</b>
<b><i>Mongoose lemurs</i></b>			
Carolina	F	04/03/10	1, 2, 3
Concepcion	F	10/15/84	1, 2, 3
Felipe	M	04/22/96	1, 2, 3
Flor	F	05/20/86	1, 2, 3
Guadalupe	F	05/26/95	1, 2, 3
Julio	M	03/19/90	1, 2, 3
Maddie	F	06/02/06	1, 2, 3
Moheli	F	05/13/87	1, 2, 3
Paco	M	06/26/97	1, 2, 3
Pedro	M	06/17/90	1, 2, 3
Sancho	M	Est. 1982	1, 2, 3
<b><i>Ringtailed lemurs</i></b>			
Aracus	M	05/23/91	1, 2, 3
Berisades	M	03/28/04	1, 2, 3
Chloris	F	04/17/88	1, 2
Fanta	F	03/23/07	3
Fresca	F	03/23/07	3
Ginger	F	04/08/06	3
Ivy	M	05/04/04	1, 2, 3
Johan	M	05/03/10	1, 2
Licinius	M	03/17/93	1, 2, 3
Liesl	F	07/15/08	3
Lilah	F	03/28/05	1, 2
Persephone	F	04/25/05	3
Randy	M	03/12/06	3
Schroeder	F	03/22/92	3
Shasta	F	05/01/08	3
Teres	M	03/23/95	2
Tugger	M	05/16/83	1, 2
Alastor	M	07/08/09	1, 2
<b><i>Coquerel's sifakas</i></b>			
Antonia	F	02/22/98	2
Brutus	M	02/28/01	1, 2
Conrad	M	12/31/08	1, 2, 3
Drusilla	F	04/06/93	3
Gaius	M	01/03/08	1, 2, 3
Irene	F	01/27/07	1, 2, 3

Julian	M	03/04/93	1, 2, 3
Lucius	M	01/07/05	1, 2, 3
Marcus	M	02/16/04	1
Martin	M	01/25/08	1, 3
Matilda	F	12/21/07	1, 2, 3
Pia	F	02/15/99	2
Pompeia	F	02/04/09	1, 3
Rhodelinda	F	08/06/07	1
Romulus	M	02/03/10	3
Rupert	M	12/15/09	2
Rupi	F	02/16/99	1, 2, 3
Tiberius	M	01/20/88	2, 3
Wilhemina	F	12/25/09	2
<b><i>Ruffed lemurs</i></b>			
Alphard	M	04/22/89	1, 2
Amor*	M	04/15/81	1, 2
Aries	M	07/02/07	2, 3
Arior	M	05/24/08	1, 2
Carina	F	05/11/04	1, 2
Eros*	M	05/22/09	1, 2, 3
Esther	F	04/29/09	1, 2, 3
Hunter	M	05/17/96	1, 2, 3
Hydra	F	05/24/08	1, 2, 3
Kizzy*	F	04/17/05	2, 3
Krok*	M	05/22/09	1, 2, 3
Lyra	F	05/24/08	2, 3
Magellan*	M	05/24/10	3
Orion Jr	M	04/29/09	1, 2, 3
Phoebe	F	04/29/09	1, 2, 3
Scorpius	M	07/02/07	1, 2, 3

**Table S1: Subject characteristics and participation across the studies.** Ruffed lemurs marked with a star (\*) are black and white ruffed (*V. variegata*), whereas the others are red ruffed (*V. rubra*).

### ***Testing periods***

Subjects were tested in study 1 between June 2010 and July 2011, in Study 2 between February 2010 and June 2011, and in Study 3 between November 2011 and December 2012. The particular lemurs tested across studies varied due to whether the individual was free-ranging, was pregnant or had a dependent offspring, or illness at the time of the study. In addition, Study 3 required that a particular lemur had access to two connected rooms for the setup. Subjects that completed both studies 1 and 2 had a minimum break of 1 month between the studies (given their similar setup), and were tested with the new apparatus in a different room or new spatial arrangement in their home-room. All testing occurred between 9 AM and 4 PM, with individual tests varying based on the schedule at the DLC.

## Study 1: Additional analysis

### *Reward manipulation*

To assess whether lemurs exhibited improved memory in certain contexts, we additionally examined the effect of reward motivation. In the current study, we manipulated reward motivation by varying the type of food that lemurs received on the last two trials of the introductory session to assess whether this influenced their subsequent memory in the test session. For the majority of trials in the introductory and test sessions, lemurs made choices about an intermediately-preferred food type, but on the last two introductory trials they received either a very high-quality food type (the positive condition) or a different intermediately-preferred food (the neutral condition). Condition assignment was counterbalanced across subjects. Food assignments were based on an initial preference pretest conducted immediately after the habituation phase, and involved the lemur making a series of 12 dichotomous choices between three different types of species-appropriate foods (ruffed lemurs, mongoose lemurs, and ringtailed lemurs were tested with fruit, and sifakas were tested with various nuts, as folivores are not regularly fed fruit at the Duke Lemur Center). During test trials the experimenters stood in the same positions as in the main sessions: E2 centered the lemur as in the memory sessions, and E1 held out their hands (approximately 6 in apart) on the tables with the two different food types to assess which one the lemurs would approach first. Side assignment and food pairings were counterbalanced across trials.

An initial analysis examining the impact of reward motivation on the lemurs' memory indicated that lemurs who experienced the positive condition chose correctly on  $M = 88.1 \pm SE = 4.9\%$  of test trials, and lemurs in the negative condition chose correctly on  $89.1 \pm 3.8\%$  of test trials [Mann Whitney U:  $Z = -0.21$ ,  $N_1 = 22$ ,  $N_2 = 22$ ,  $p = 0.83$ , n.s.]. There was also no difference in first trial performance across conditions, with 17/22 individuals in the positive condition choosing correctly on their first test trial, and 16/22 individuals in the neutral condition choosing correctly on their first test trial [ $\chi^2 = 0.12$ ,  $df = 1$ ,  $N = 44$ ,  $p = 0.73$ , n.s.]. As there was therefore no evidence that the reward manipulation influenced memory performance, possibly because the experimental manipulation involved only a slight difference in reward value across the two conditions We therefore collapsed across reward motivation conditions for all further analyses.

### *Generalized linear mixed model examining test trial performance*

<i>Factor</i>	<i>Estimate</i>	<i>S.E.</i>	<i>P</i>
Trial	0.413	0.0859	< <b>0.001</b>
Introductory mean	6.971	2.521	< <b>0.01</b>
Species (Ruffed)	2.121	1.093	<b>0.052</b>

**Table S1: Factors influencing the lemurs' likelihood for making a correct choice on test trials in Study 1.** The full generalized linear mixed model assessed the influence of the following factors: trial number (1-10), performance in the introductory session (proportion correct in the initial learning trials), and species (ruffed compared to other species).

## Study 2: Additional analysis

### *Ruffed lemur performance*

In study 2, two ruffed lemurs were tested with a slightly modified baiting procedure (see Table S1). For these individuals, E2 stood outside the room and attracted the lemur to floor while E1 baited the cups out of the lemur's view. E1 then attracted the lemur to the normal starting position. The main choice phase of each trial proceeded as normal. To ensure that the inclusion of the individuals did not influence the results, we examined the performance of the other 13 ruffed lemurs who were tested in the standard fashion. These individuals choose the correct option on  $94.3 \pm 9.5$  % of learning trials, significantly above chance [ $t_{12} = 47.34$ ,  $p < 0.001$ ]. In probe trials, these 13 ruffed lemurs chose the spatial option on  $78.2 \pm 4.8$ % of trials, also above chance [ $t_{12} = 5.915$ ,  $p < 0.001$ ]. Finally, this subset of ruffed lemurs showed a preference for the spatial option in the first probe trial [binomial test, 11 of 13 chose the spatial option,  $p < 0.05$ ]. Overall, these results align with the analysis including all 15 ruffed lemurs that were reported in the main test. This indicates that these two individuals performed similarly to the other ruffed lemurs, despite the slight modification to their testing procedure.

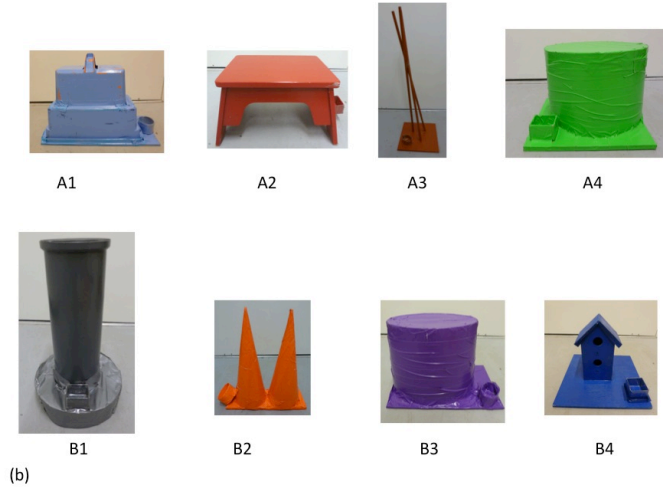
### *Generalized linear mixed model examining probe trial performance*

<i>Factor</i>	<i>Estimate</i>	<i>S.E.</i>	<i>P</i>
Session	0.039	0.088	0.66
Learning Mean	-1.396	1.236	0.26
Species (Ruffed)	1.247	0.425	<b>&lt;0.005</b>

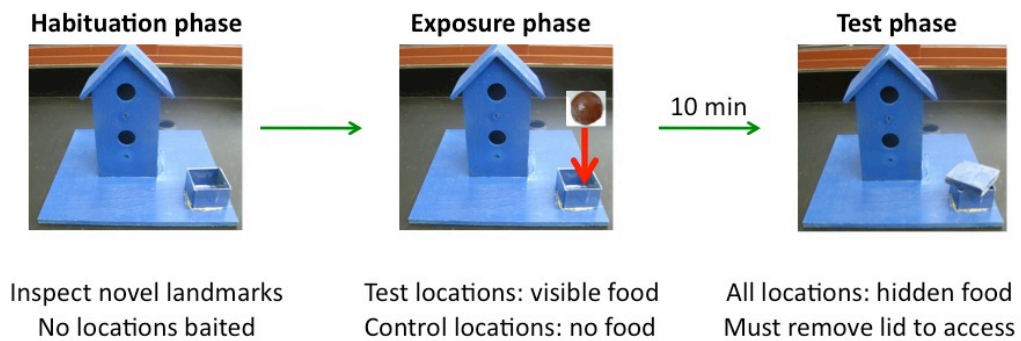
**Table S2: Factors influencing the lemurs' likelihood for making an spatial response on probe trials in Study 2.** The full generalized linear mixed model assessed the influence of the following factors: session number (1-6), learning trial performance (proportion correct in the preceding learning trials that session), and species (ruffed compared to other species).

## Study 3: Landmarks

We used eight distinct landmarks for hiding the food (see Figure S1). These landmarks were: A1) light blue stacked boxes A2): a red stool; A3) three upright brown wooden dowels; A4) a textured green column; B1) a tall grey column; B2) two attached orange cones; B3) a wide purple column; and B4) a dark blue birdhouse. The landmarks were placed in an approximately identical arrangement on the floor and on the shelves for each subject in order to control for location biases. For most lemurs, landmarks A1 and B3 were placed on two shelved in their room (at a height of 3 ft to 5 ft across rooms); the rest were located on the ground. For 2 lemurs, landmarks A1 and B3 were placed on the ground in the equivalent location, as their room lacked shelves. Across phases of the session, the landmark baiting boxes varied (see Figure S2). In the exposure phase (in which lemurs could acclimate to the novel objects), all locations were visibly empty. In the exposure phase, test locations were visibly baited (test locations in exposure phase), and control locations were empty. Finally, in the search phase all locations were baited with food, but the food was covered with an easily removable lid.



**Figure S1: Landmarks and room setup used in study 3.** (Each landmark had a unique color and shape. A small box was attached to each landmark, which could be baited with food.)



**Figure S2: Baiting procedure in study 3.** In the habituation phase, lemurs could investigate the novel landmarks. In the exposure phase, test locations only were baited with visible food. In the search phase, all locations were baited, but the food was hidden as the food boxes were covered by removable lids.