



Published in final edited form as:

Primates. 2014 January ; 55(1): 25–34. doi:10.1007/s10329-013-0361-7.

Variation in hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in long-tailed macaques (*Macaca fascicularis*) from Singapore

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Abstract

Much of the primatology literature on stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) has focused on African and New World species, with comparatively little research published on Asian primates. Here we present hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values for a sample of 33 long-tailed macaques from Singapore. We evaluate the suggestion by a previous researcher that forest degradation and biodiversity loss in Singapore have led to a decline in macaque trophic level. The results of our analysis indicated significant spatial variability in $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$. The range of variation in $\delta^{13}\text{C}$ was consistent with a diet based on C_3 resources, with one group exhibiting low values consistent with a closed canopy environment. Relative to other macaque species from Europe and Asia, the macaques from Singapore exhibited a low mean $\delta^{13}\text{C}$ value but mid-range mean $\delta^{15}\text{N}$ value. Previous research suggesting a decline in macaque trophic level is not supported by the results of our study.

Keywords

Stable isotopes; $\delta^{13}\text{C}$; $\delta^{15}\text{N}$; Primates; Macaques; *Macaca fascicularis*; Singapore

Introduction

Analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios has become an important avenue of research for primatologists interested in the study of feeding ecology and nutrition in nonhuman primates (see Crowley 2012; Sandberg et al. 2012). Until only recently, much of the research on stable isotopes in nonhuman primates had focused on African and New World primates (see Schoeninger 2010), but not on Asian primates such as macaques. In the following report we describe intra- and intergroup variation in stable isotope ratios in a population of long-tailed macaques in Singapore. Specifically, we examine recent research by Gibson (2011) suggesting there has been a significant and detectable shift in the macaque trophic level in Singapore due to habitat modification and biodiversity loss associated with infrastructure development. In addition, we place the observed variation in stable isotope data within a larger context for the genus *Macaca*.

Singapore is highly urbanized, with less than 5 % of its land area (714.3 km²) constituting protected forests (see discussions in Davison et al. 2012; Brook et al. 2003) under the protection of the National Parks Board, Singapore. Despite being urbanized, Singapore is home to three genera of primates (*Macaca*, *Presbytis*, *Nycticebus*). Among these, the long-tailed macaque [*Macaca fascicularis* (Raffles 1821)] is by far the most numerous. Long-tailed macaques are small- to medium-sized frugivorous/omnivorous monkeys with a wide distribution across much of peninsular Southeast Asia, Indonesia, and portions of the Philippines (Fooden 1982). A census conducted in Singapore in 2007 (Sha et al. 2009a) estimated there were between 1,218 and 1,454 macaques on the entire island (also see Agoramoorthy and Hsu 2006). Singapore's remaining large green spaces (i.e., nondeveloped space) consist of nature reserves with both primary and secondary forest fragments, anthropogenic heath forests, and regional urban parks. About 70 % of the island's macaque populations reside in Bukit Timah and Central Catchment Nature Reserves, which comprise a series of forests and reservoirs located near the center of the island (Sha et al. 2009a,b).

Background on stable isotopes

Stable isotope analysis of carbon (^{12}C and ^{13}C) and nitrogen (^{14}N and ^{15}N) in animal tissues can provide valuable information on diet. Plants utilizing the C_3 -photosynthetic pathway (e.g., trees, shrubs, and forbs) are depleted in ^{13}C relative to C_4 -photosynthetic (e.g., grasses) and crassulacean acid metabolism (CAM) (e.g., succulents) plants (Codron et al. 2006). The ratio of heavy to light isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) in animal tissue will vary according to the relative contribution of C_3 and C_4 plants in an animal's diet. This ratio is measured in δ notation as parts per thousand deviation or ‰, relative to internationally recognized standards (Crowley et al. 2011; see Schoeninger 2010 for review). The relative proportion of carbon isotopes ($\delta^{13}\text{C}$) varies from −35 to −21 ‰ in C_3 plants, −20 to −10 ‰ in C_4 plants, and −17 to −13.2 ‰ in CAM plants (see O'Leary 1981, 1988; Kohn 2010). In the tropics, variation in $\delta^{13}\text{C}$ values in C_3 plants can sometimes be attributed to higher

concentrations of atmospheric carbon dioxide associated with more closed forest canopies (Medina and Minchin 1980; van der Merwe and Medina 1989; Schoeninger 2010; Crowley et al. 2012). Because the degree of forest canopy and the distribution of C_3 and C_4 plants can vary spatially, variation in $\delta^{13}C$ values within and among animal groupings may reflect differences in feeding ecology, differences in geographic location, or both.

Stable nitrogen isotope ratios ($^{15}N/^{14}N$) are useful for investigating trophic relationships within animal communities because consumer $\delta^{15}N$ values predictably increase with increased faunivory (DeNiro and Epstein 1981; Schoeninger and DeNiro 1984). C_3 plants with symbiotic nitrogen-fixing bacteria have $\delta^{15}N$ values close to 0 ‰ (Virginia and Delwiche 1982). Herbivores have $\delta^{15}N$ values ranging between 3 and 5 ‰ higher than the plants they consume, while the consumers of herbivores have $\delta^{15}N$ values 3–5 ‰ higher than the herbivores they consume (DeNiro and Epstein 1981; Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Crowley et al. 2011). There are other potential contributors to variation in $\delta^{15}N$ values; For example, fruit may have higher $\delta^{15}N$ values than leaves, and forest disturbance may lead to increased stable nitrogen isotope ratios in generalists such as rodents and primates (Nakagawa et al. 2007; Gibson 2011). In addition, fertilizer use and sea spray can influence $\delta^{15}N$ values in plants. Stress leading to a catabolic state can also increase an animal's $\delta^{15}N$ values (Hobson and Clark 1992; Hobson et al. 1993; Crowley et al. 2011).

Previous research

Although much of the literature on stable isotope ratios in nonhuman primate hair has focused on chimpanzees, New World monkeys, and lemurs (Schoeninger et al. 1997, 1998, 1999; Sponheimer et al. 2006; Loudon et al. 2007; Schoeninger 2010), recent research has begun to examine variation in macaques (O'Regan et al. 2008; Engel et al. 2010; Gibson 2011; Schurr et al. 2012). For their research on dietary heterogeneity in *M. mulatta*, O'Regan et al. (2008) compared stable isotope ratios of carbon and nitrogen in hair and bone samples taken from a geographically diverse collection of museum specimens representing both tropical and temperate populations. The authors of that study found strong correlations in stable isotope ratios between hair and bone tissues. Their results suggested that, while the hair samples from the tropical populations indicated a diet based on C_3 plants, the samples taken from temperate populations from higher latitudes indicated that some C_4 -based resources were consumed. In their analysis of stable isotope ratios in *M. mulatta* from Nepal, Engel et al. (2010) found a significant difference between older and younger monkeys in mean $\delta^{15}N$, as well as a significant difference across groups in $\delta^{13}C$. More recently, Schurr et al. (2012) examined intergroup variation and the effects of tourism/provisioning on stable isotope ratio values in *M. sylvanus* from Gibraltar. The authors of that study found significant differences in $\delta^{13}C$ and $\delta^{15}N$ values between a group with minimal contact with tourists and those groups with greater exposure to tourists. Provisioning by tourists was suggested as the likely source of intergroup differences in stable isotope values (Schurr et al. 2012).

Variation in hair $\delta^{13}C$ and $\delta^{15}N$ values in macaques from Singapore has been studied previously. Gibson (2011) analyzed hair samples taken from both living monkeys and

Primates. Author manuscript; available in PMC 2015 May 28.

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where X represents ^{13}C or ^{15}N , in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R represents $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, for samples and standards, respectively. Isotopic data were normalized to V-PDB and AIR using the primary standards USGS 40 (−26.24 and −4.52 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) and USGS 41 (37.76 and 47.57 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). Analytical error was assessed by replicate measures of primary standards (<0.2 ‰ across all analytical sequences) and quality control assessed using several secondary standards analyzed several times within individual analytical sequences (<0.3 ‰); accuracy was assessed using primary standards as unknowns and was within 0.2 ‰ for all isotopes.

For comparative purposes data on stable isotope ratios from hair for *Macaca mulatta* and *M. sylvanus* were taken from O'Regan et al. (2008), Engel et al. (2010), and Schurr et al. (2012). Additional data for *M. fascicularis* from Singapore were taken from Gibson (2011). Statistical comparisons of analytical groupings were achieved using standard t tests after testing the assumptions of normality and equality of variance. When the assumption of normality was not met, a nonparametric Mann–Whitney U test was used. A significance level of $\alpha = 0.05$ was used for all tests. All p values are two-tailed unless otherwise indicated.

Results

The results of our analysis of stable carbon and nitrogen isotope ratios in macaque hair are presented in Table 1. Stable isotope ratios for carbon, but not nitrogen, varied largely by geographic location rather than by sex or age (Table 2). Statistical comparisons across locations were limited by sample sizes. Significant differences in mean $\delta^{13}\text{C}$ values were detected between Bukit Timah and the end of Rifle Range Road (Mann–Whitney U , $p = 0.004$) and between Bukit Timah and Upper Seletar (Mann–Whitney U , $p = 0.005$). Comparisons of male and female stable isotope ratios failed to reveal significant differences in either $\delta^{13}\text{C}$ ($t = 1.263$, $p = 0.216$) or $\delta^{15}\text{N}$ ($t = 1.197$, $p = 0.240$). Although small sample sizes precluded analysis of variance, age categories exhibited similar distributions of stable isotope values (Fig. 2a, b). Comparisons between the two age categories with the largest sample sizes indicated no significant difference between juveniles and adults for either $\delta^{13}\text{C}$ ($t = 1.107$, $p = 0.280$) or $\delta^{15}\text{N}$ ($t = 1.019$, $p = 0.318$).

With the exception of Bukit Timah, $\delta^{13}\text{C}$ values across locations within Singapore ranged between −22 and −24 ‰ (mean = −23.2 ‰, SD = 0.84, $n = 33$) (Fig. 3a). Most of the values (7 of 9) for Bukit Timah were less than −24 ‰. Among the locations sampled, Bukit Timah exhibited the lowest mean $\delta^{13}\text{C}$ value (mean = −24.0 ‰). The highest mean $\delta^{13}\text{C}$ value was exhibited by monkeys trapped at the end of Rifle Range Road (mean = −22.5 ‰). The average difference in mean $\delta^{13}\text{C}$ among sample locations (21 comparisons across 7 locations) in Singapore (0.7 ‰) differed significantly ($t = 3.00$, two-tailed, $p = 0.005$) from the average difference among sample locations reported for the macaques of Gibraltar (mean = 0.2 ‰, 10 comparisons across 5 locations). The average difference in mean $\delta^{13}\text{C}$ among

sample locations in Nepal (mean = 0.5 ‰, 3 comparisons) was between that observed for Gibraltar and Singapore.

The distribution of individual $\delta^{15}\text{N}$ values for each group is presented in Fig. 3b. Group averages for stable nitrogen isotope ratios varied from 5.6 ‰ midway down Rifle Range Road to 3.8 ‰ at Bukit Kalang (Table 2). None of the comparisons among locations with sufficient sample sizes (i.e., Bukit Timah, end of Rifle Range Road, Upper Seletar) revealed a significant difference in mean $\delta^{15}\text{N}$ values. We compared the historical sample values reported by Gibson (2011) with a modern sample comprising stable nitrogen ratio values generated by our analysis and those presented by Gibson (2011) for 2009. All but two historical values (1943 and 1944) fell within 2 standard deviations of the modern sample. It is important to note that the 1943 value presented by Gibson (2011) represents an infant who may have been nursing, which likely results in enriched $\delta^{15}\text{N}$ values (see Engel et al. 2010). Interestingly, the highest $\delta^{15}\text{N}$ obtained by our study also came from an infant who likely was still in the process of weaning. The average difference in mean $\delta^{15}\text{N}$ among sample locations in Singapore (mean = 0.7 ‰, 21 comparisons across 7 locations) did not differ significantly ($t = 1.224$, $p = 0.226$) from the average difference among sample locations reported for the macaques of Gibraltar (mean = 0.5 ‰, 10 comparisons across 5 locations). The average difference in mean $\delta^{15}\text{N}$ among sample locations in Nepal (mean = 0.1 ‰, 3 comparisons) was small compared with that observed for Gibraltar and Singapore.

The long-tailed macaques from Singapore, along with the rhesus macaques from northern Vietnam and Burma, exhibited a low mean $\delta^{13}\text{C}$ in our genus-wide comparison of stable isotope ratio values (Fig. 4). The mean $\delta^{13}\text{C}$ value from the Singapore macaques ($n = 33$, mean = -23.2 , SD = 0.83) was significantly lower ($t = 9.316$, $p < 0.001$) than the mean value for the Gibraltar macaques ($n = 88$, mean = -21.9 , SD = 0.57) and the macaques from Nepal ($n = 37$, mean = -21.7 , SD = 0.72). The low values from the Singapore macaques are consistent with a diet based on C_3 resources in a tropical environment (O'Regan et al. 2008). Although small sample sizes preclude statistical comparison, the macaques from Arunchal Pradesh ($n = 2$, mean = -20.6) and Uttar Pradesh ($n = 5$, mean = -17.4) in northern India exhibited substantially higher mean $\delta^{13}\text{C}$ values. The higher mean values from northern India invite speculation that these monkeys from more temperate climates included some C_4 resources in their diet (O'Regan et al. 2008). The Barbary macaques (*M. sylvanus*) from Gibraltar exhibited mean $\delta^{13}\text{C}$ values intermediate to those of the tropical macaques feeding on C_3 resources and those from more temperate climates that may feed on some C_4 resources. Schurr et al. (2012) indicated that, of the macaque groups that they studied in Gibraltar, only the monkeys from Middle Hill are not fed by tourists, though approximately 50 % of their diet consists of provisioned foods. Of the natural plant resources eaten by the Middle Hill group, almost all are C_3 plants (Schurr et al. 2012). Parenthetically, recent experimental research (Nakashita et al. 2013) comparing hair $\delta^{13}\text{C}$ values in macaques (*M. fuscata*) fed different diets found increased average $\delta^{13}\text{C}$ values ($\delta^{13}\text{C} = 2.8 \pm 0.3$) in the hair of individuals fed a diet heavily enriched with corn (i.e., C_4 diet).

The mean $\delta^{15}\text{N}$ value for the macaques from Singapore ($n = 33$, mean = 4.9, SD 0.77) lies near the middle of the distribution of mean values for *M. mulatta*. The mean for the Singapore macaques is significantly lower ($t = 3.28$, $p = 0.002$) than the mean for the

Gibraltar macaques ($n = 88$, mean = 5.5, SD = 0.83), and significantly lower ($t = 2.05$, $p = 0.044$) than the macaques from Nepal ($n = 37$, mean = 5.2, SD = 0.52). Interestingly, all of the mean values greater than the Singapore macaques come from populations that are either provisioned or receive food from tourists, or forage from human refuse (i.e., Gibraltar and Nepal) (Engel et al. 2010; Schurr et al. 2012). All of the mean $\delta^{15}\text{N}$ values that are less than the Singapore population, however, are from presumably nonprovisioned wild macaque groups.

Discussion

Our analysis of stable isotope ratios in macaque hair from Singapore revealed a range of variation in $\delta^{13}\text{C}$ consistent with a diet based on C_3 resources. Using comparative research on $\delta^{13}\text{C}$ in nonhuman primate hair presented by Schoeninger (2010, Fig. 15.3), we established a threshold value of -24‰ to identify a closed canopy environment (but see Crowley et al. 2012). With the exception of one individual from Rifle Range Road, all hair samples with $\delta^{13}\text{C} \leq -24\text{‰}$ were from monkeys from Bukit Timah. Hair samples from other locations exhibited $\delta^{13}\text{C}$ greater than this threshold, indicative of more open environments (Schoeninger 2010; O'Regan et al. 2008; Schoeninger et al. 1997), such as those adjacent to reservoirs within the Central Catchment Nature Reserve of Singapore.

Based on the significantly larger mean among group difference, the Singapore macaques exhibit a higher level of dietary heterogeneity as reflected by stable carbon and nitrogen isotope ratios than the macaques from Gibraltar and Nepal—the only other macaques for which intergroup differences have been reported (Engel et al. 2010; Schurr et al. 2012). It is important to point out that the data from Nepal are derived from only three groups in close geographic proximity. Our inference is supported by a larger coefficient of variation for $\delta^{13}\text{C}$ observed for the Singapore macaques ($\text{CV} = 3.61$, $n = 33$) compared with the macaques from Gibraltar ($\text{CV} = 2.59$, $n = 88$) and Nepal ($\text{CV} = 2.41$, $n = 37$). The relative variation in stable nitrogen isotope ratio values was similarly higher for the Singapore macaques ($\text{CV} = 15.69$) compared with the macaques from Gibraltar ($\text{CV} = 14.95$) and Nepal ($\text{CV} = 13.69$). Importantly, however, our inference regarding dietary heterogeneity is limited by the lack of baseline data on stable isotope values in the food consumed by the macaques.

Our genus-wide comparison revealed a relatively low mean $\delta^{13}\text{C}$ for the Singapore macaques (mean = -23.2 , SD = 0.836), consistent with other tropical macaques that rely on C_3 plant resources. Relative to the macaques from Gibraltar and Nepal, the Singapore macaques exhibited a lower mean $\delta^{15}\text{N}$ value (mean = 4.9, SD = 0.77). Relative to the macaques from Burma and Gujarat, India, however, the Singapore macaques exhibited a higher mean $\delta^{15}\text{N}$ value. The lower $\delta^{15}\text{N}$ in the Burmese and Gujarat monkeys may be a product of diet which included a significant amount of legumes (see O'Regan et al. 2008). Schurr et al. (2012) suggested that macaque exposure to tourists who feed monkeys in Gibraltar is associated with lower $\delta^{15}\text{N}$ values, as commercially fertilized plant foods used for provisioning have lower $\delta^{15}\text{N}$ values than natural plants. The Singapore macaques exhibited a high mean $\delta^{15}\text{N}$ value relative to other wild macaque populations included from India, but a lower mean than the provisioned macaques from Gibraltar, and the macaques

from Nepal that are exposed to tourists and who feed on local refuse (Engel et al. 2010). Macaques in Singapore are sometimes fed by local residents and park visitors, and will also raid residential trash cans (Sha et al. 2009b; Lee and Chan 2011). It is difficult to determine, therefore, if the difference in $\delta^{15}\text{N}$ values between the wild macaques and those that are provisioned and/or are exposed to tourists is a product of feeding on leguminous food resources (reduced $\delta^{15}\text{N}$) or exposure to provisioning and human food. It is important to recognize that, in principle, given the large geographic separation of the groups being compared, complexity in nitrogen cycles could contribute to the observed differences in $\delta^{15}\text{N}$ values even if these groups were feeding on the same plant foods. In any case, the mean $\delta^{15}\text{N}$ for the macaques from Singapore is placed well within the trophic spectrum for the genus.

Recently, Gibson (2011) used stable isotope ratio analysis to compare the diets of two temporal groupings of long-tailed macaques in Singapore. The first group comprised museum specimens dating to between 1910 and 1944 ($n = 6$). The second group comprised living macaques trapped and sampled in 2009 ($n = 8$). A comparison of $\delta^{15}\text{N}$ values revealed a significant difference between groups, with the historical group exhibiting a greater average (mean = 4.3 ‰) than the modern group (mean = 3.9 ‰). Gibson (2011) interpreted this significant difference as evidence of a trophic level shift in macaque feeding associated with deforestation. Parenthetically, it is worth noting, as did Gibson (2011, p. 218), that the $\delta^{15}\text{N}$ value for a macaque collected in 1910 (−3.2 ‰) is likely an unreliable measure of the relative proportion of nitrogen isotopes in Gibson's study. Without this individual the mean for the historical group increases to 5.8 ‰. When the results of the present study are combined with Gibson's (2011) modern sample, the average increases from a mean of 3.9 ‰ to a mean of 4.7 ‰. This new average is still significantly lower ($t = 2.670$, single-tailed $p = 0.005$) than the historical sample average as recalculated (i.e., 4.7 ‰ < 5.8 ‰).

On the surface, the comparison above would seem to support Gibson's hypothesis regarding a trophic shift. However, if we examine $\delta^{15}\text{N}$ values by year we see that all the historical values fall within the range of variation of the modern sample (Fig. 5). In addition, because maternal milk can enrich $\delta^{15}\text{N}$ in the tissues in nursing infants (Jenkins et al. 2001; Reitsema 2012), close attention should be paid to infants and young juveniles undergoing the weaning process. If we exclude likely nursing juveniles from both groups, then only the value for 1944 exhibits a high $\delta^{15}\text{N}$ value relative to the distribution of modern values. A comparison of $\delta^{15}\text{N}$ values by year reveals a trophic increase between 1910 and 1944, a period after widespread deforestation was almost completed on Singapore (Corlett 1992). Such an increase, albeit based on only 5 values, is inconsistent with the hypothesis that deforestation and biodiversity loss have resulted in a trophic shift for Singapore's macaques. Furthermore, our study has shown that the distribution of hair $\delta^{15}\text{N}$ values varies considerably among macaque groups within a relatively small geographic region, with group averages ranging from a low mean of 3.8 ‰ for the Bukit Kalang group to a high mean of 5.6 ‰ for the group located midway along Rifle Range Road. It is important to note that the observed differences among groups in our study were not significant statistically, and a number of groups had low sample sizes precluding formal comparisons.¹ If the observed difference between Bukit Kalang ($n = 3$) and the group located midway along Rifle Range Road ($n = 4$) is not a

statistical artifact stemming from small sample sizes, then this difference (i.e., 1.8 ‰) represents 0.5 trophic level difference across macaque groups. Drawing samples from only three macaque groups in modern times, as Gibson (2011) did for his study, may not provide a reliable estimate of the mean $\delta^{15}\text{N}$ for the macaque population on the island. In fact, the mean $\delta^{15}\text{N}$ for our study which included hair samples from 33 macaques from 7 different locations (mean = 4.9 ‰) was significantly different ($t = 3.380$, two-tailed $p = 0.002$) from the average of the modern macaques included in Gibson's study (mean = 3.9 ‰) which consisted of 8 individuals from only 3 locations. It seems reasonable to suggest, therefore, that the high and low mean $\delta^{15}\text{N}$ values observed for the historical and modern groups in Gibson's study may be subject to sampling error. In addition, Gibson's study, like ours, is limited by not including an analysis of $\delta^{15}\text{N}$ values in the plants eaten by macaques. The macaques in Singapore eat a wide variety of foods with differing $\delta^{15}\text{N}$ values. Because macaque diets will vary by geographic location within Singapore (see Sha and Hanya 2013), more comprehensive knowledge of how this dietary heterogeneity might affect the geographic distribution of $\delta^{15}\text{N}$ values in macaque hair is needed before making inferences regarding trophic shifts through time. Furthermore, nitrogen cycles are complex and can be influenced by a number of factors not necessarily related to forest degradation and biodiversity loss associated with urbanization, such as local geology, rainfall, and changes in fertilizer use.

In conclusion, our analysis of stable isotope ratios revealed significant variability among geographic locations in $\delta^{13}\text{C}$ but not $\delta^{15}\text{N}$. The range of variation in $\delta^{13}\text{C}$ was consistent with a diet based on C_3 resources, with the group from Bukit Timah exhibiting a low value indicative of a closed canopy environment. Relative to other macaque species from Europe and Asia, the macaques from Singapore exhibit a low mean $\delta^{13}\text{C}$ value but mid-range mean $\delta^{15}\text{N}$ value. Previous research by Gibson (2011) on stable isotope ratios in historical and modern macaque hair samples from Singapore suggesting a trophic shift in macaque feeding associated with deforestation and biodiversity loss is not supported by the present study. Future research should include sampling of plants known to be eaten by the macaques studied, as well as analysis of a sufficient number of hair samples collected from multiple geographic groupings.

Acknowledgments

We thank the National Parks Board (Singapore), especially Sharon Chan, for supporting the project. We thank Dr. James Loudon and Dr. Mark Schurr for their insightful comments and criticisms of earlier drafts. Trapping and data-collection procedures used for this study were approved by the institutional animal care and use committee of the University of Toronto (protocol no. 20005356). Partial funding for this project was provided by the University of Toronto Scarborough, and the University of Toronto Connaught Fund (awarded to M.A.S.). Thanks are due to Cayce Gulbransen (U.S. Geological Survey) for conducting the stable isotope analyses. The content of this paper is solely the responsibility of the authors and does not necessarily represent the views of our funders. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

¹Recent research has indicated that with random sampling the minimum sample size needed to estimate the population mean is $n = 5$ (Schillaci and Schillaci 2009).

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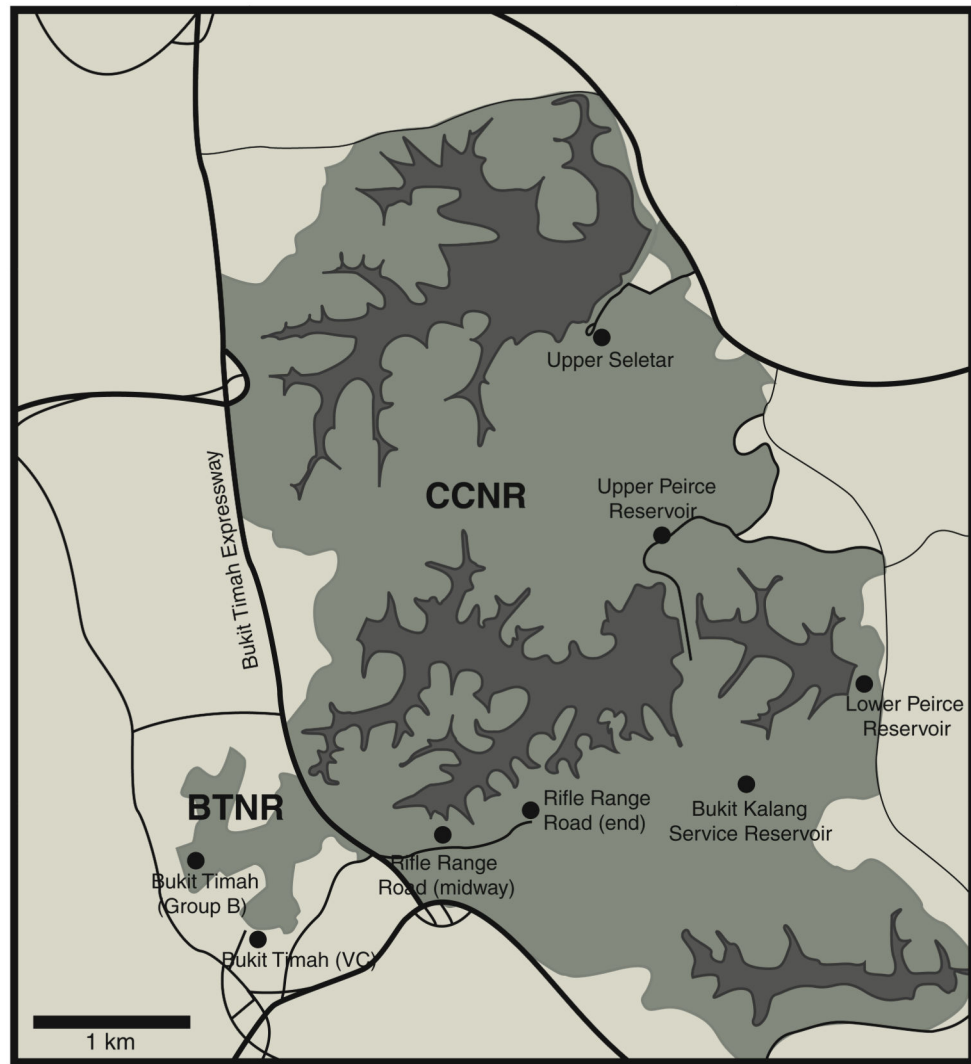


Fig. 1.
Map of the study area showing trapping locations. Figure taken from Schillaci et al. (2011a, b)

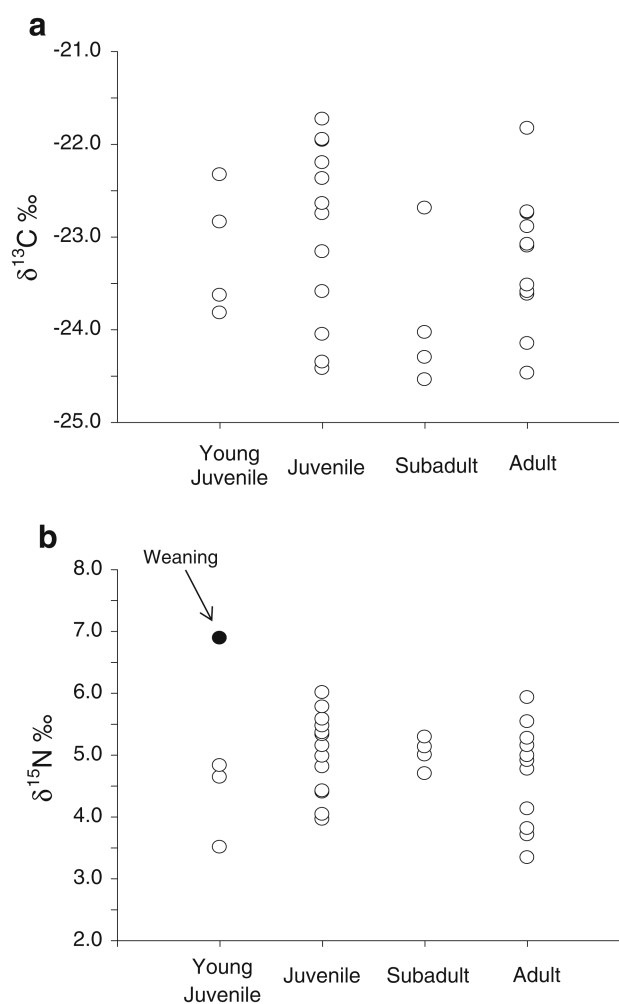


Fig. 2. Dot plot of (a) stable carbon ($\delta^{13}\text{C}$ ‰) and (b) stable nitrogen ($\delta^{15}\text{N}$ ‰) isotope ratios by age category

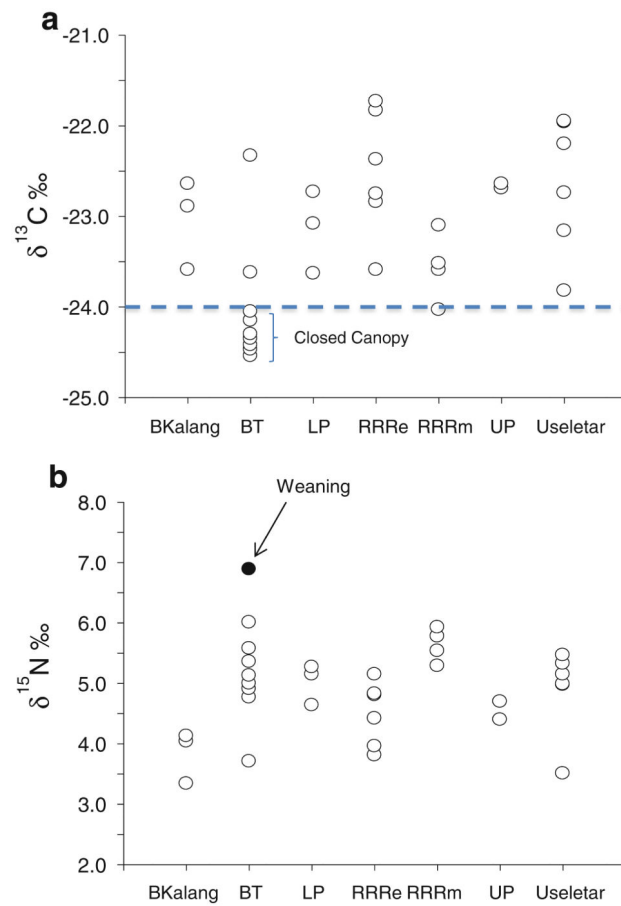


Fig. 3. Dot plot of (a) stable carbon ($\delta^{13}\text{C} \text{ ‰}$) and (b) stable nitrogen ($\delta^{15}\text{N} \text{ ‰}$) isotope ratios by trapping location. *BKajang* Bukit Kalang, *BT* Bukit Timah, *LP* Lower Peirce Reservoir, *RRRe* Rifle Range Road (end), *RRRm* Rifle Range Road (midway), *UP* Upper Peirce Reservoir, *Useletar* Upper Seletar

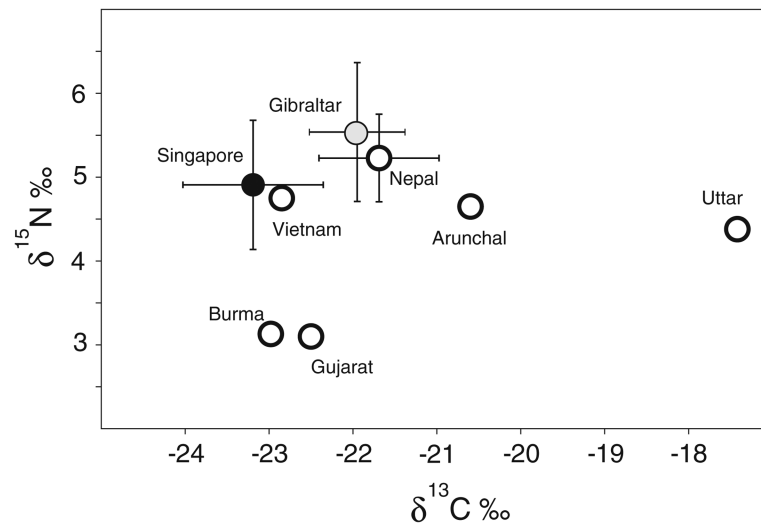


Fig. 4. Plot of mean stable carbon ($\delta^{13}\text{C} \text{ ‰}$) and stable nitrogen ($\delta^{15}\text{N} \text{ ‰}$) isotope ratios for the genus *Macaca*. Error bars describe 1 standard deviation. Open circles, *M. mulatta*; gray-filled circle, *M. sylvanus*; black-filled circle, *M. fascicularis*

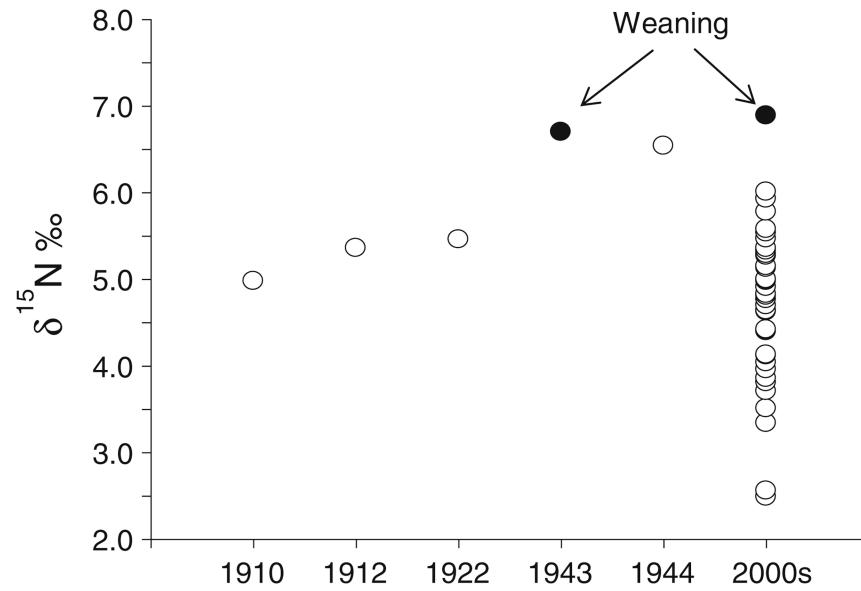


Fig. 5.

Dot plot of stable nitrogen ($\delta^{15}\text{N}$ ‰) isotope values by year. The historical values do not include outlier discussed in text. Sample values for 2000s include 8 values presented in Gibson (2011) for 2009, as well as values for macaques sampled in 2003 and 2005 for this study

Table 1

Results of stable isotope analysis

ID	Location ^a	Year	Sex	Age category ^b	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
SM19	Bukit Kalang	2005	M	Adult	-22.9	4.1
SM20	Bukit Kalang	2005	F	Adult	-23.6	3.3
SM22	Bukit Kalang	2005	F	Juvenile	-22.6	4.0
S18	Bukit Timah	2003	F	Juvenile	-24.4	5.6
S19	Bukit Timah	2003	F	Subadult	-24.3	5.1
S31	Bukit Timah	2003	F	Adult	-24.2	4.8
S33	Bukit Timah	2003	F	Adult	-24.5	4.9
S35	Bukit Timah	2003	M	Juvenile	-24.1	6.0
S37	Bukit Timah	2003	F	Juvenile	-24.4	5.4
S38	Bukit Timah	2003	M	Subadult	-24.5	5.0
S9	Bukit Timah	2003	M	Adult	-23.6	3.7
SM3	Bukit Timah	2005	F	Young juvenile	-22.3	6.9
SM14	Lower Peirce	2005	M	Adult	-23.1	5.3
SM33	Lower Peirce	2005	M	Adult	-22.7	5.2
SM37	Lower Peirce	2005	M	Young juvenile	-23.6	4.6
S26	Rifle Range Road (end)	2003	M	Adult	-21.8	5.2
S27	Rifle Range Road (end)	2003	M	Young juvenile	-22.8	4.8
S28	Rifle Range Road (end)	2003	M	Juvenile	-22.8	4.0
S29	Rifle Range Road (end)	2003	M	Adult	-23.6	3.8
S30	Rifle Range Road (end)	2003	F	Juvenile	-22.4	4.4
SM9	Rifle Range Road (end)	2005	M	Juvenile	-21.7	4.8
S21	Rifle Range Road (mid)	2003	F	Juvenile	-23.6	5.8
S23	Rifle Range Road (mid)	2003	M	Subadult	-24.0	5.3
S24	Rifle Range Road (mid)	2003	F	Adult	-23.1	5.5
S25	Rifle Range Road (mid)	2003	M	Adult	-23.5	5.9
SM12	Upper Peirce	2005	M	Subadult	-22.7	4.7
SM13	Upper Peirce	2005	M	Juvenile	-22.6	4.4
SM23	Upper Seletar	2005	M	Adult	-22.7	5.0

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ID	Location ^a	Year	Sex	Age category ^b	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
SM24	Upper Seletar	2005	F	Juvenile	-23.2	5.2
SM27	Upper Seletar	2005	M	Juvenile	-22.2	5.3
SM29	Upper Seletar	2005	M	Juvenile	-22.0	5.0
SM30	Upper Seletar	2005	F	Juvenile	-22.0	5.5
SM32	Upper Seletar	2005	M	Young juvenile	-23.8	3.5

^a See Fig. 1 for location

^b Age determined by dental eruption schedule (Schillaci et al. 2007). Young juveniles 0–12 months; male juveniles 12–39 months, female juveniles 12–36 months; male subadults 39–55 months, female subadults 36–50 months; male adults >55 months, female adults >50 months

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Table 2
Mean stable carbon and nitrogen isotope ratios by sex, age category, and location

	N	$\delta^{13}\text{C} \text{ } \text{‰} (\pm\text{SD})$	$\delta^{15}\text{N} \text{ } \text{‰} (\pm\text{SD})$
Sex			
Males	20	-23.0 (0.8)	4.8 (0.7)
Females	13	-23.4 (0.9)	5.1 (0.9)
Age category			
Young juveniles	4	-23.2 (0.7)	5.0 (1.4)
Juveniles	13	-22.9 (0.9)	5.0 (0.7)
Subadults	4	-23.9 (0.8)	5.0 (0.3)
Adults	12	-23.3 (0.7)	4.7 (0.8)
Location			
Bukit Kalang	3	-23.0 (0.5)	3.8 (0.4)
Bukit Timah	9	-24.0 (0.7)	5.3 (0.9)
Lower Peirce Reservoir	3	-23.1 (0.5)	5.0 (0.3)
Rifle Range Road (end)	6	-22.5 (0.7)	4.5 (0.5)
Rifle Range Road (mid)	4	-23.6 (0.4)	5.6 (0.3)
Upper Peirce Reservoir	2	-22.7 (0.0)	4.6 (0.2)
Upper Seletar	6	-22.6 (0.7)	4.9 (0.7)