ORIGINAL ARTICLE

Sensory integration during foraging: the importance of fruit hardness, colour, and odour to brown lemurs

Kim Valenta[1,2] · Kevin A. Brown[3] · Radoniaina R. Rafaliarison[4] · Sarah A. Styler[5] ·
Derek Jackson[6] · Shawn M. Lehman[1] · Colin A. Chapman[2,7] · Amanda D. Melin[8]

Abstract Animal reliance on fruit signals, such as hardness, colour, and odour, during foraging is poorly understood. Here, we present data on fruit foraging behaviour and efficiency (rate of fruit ingestion) of three groups of wild, frugivorous brown lemurs (*Eulemur fulvus*, $N=29$ individuals) in Ankarafantsika National Park, Madagascar. We quantify fruit hardness using a modified force gauge, fruit colour using spectroscopy, and fruit odour using volatile organic compound (VOC) sampling with gas chromatography-mass spectrometry. We relate lemur foraging behaviour to fruit traits by calculating touching, visual inspection, and sniffing indices and relate lemur foraging efficiency to fruit traits by calculating acceptance indices. The use of different sensory modalities by lemurs is marginally predicted in one case by fruit traits—fruits with higher overall smell signals are sniffed less than fruits with lower overall smell signals. When controlling for all fruit traits, fruit size is the only significant predictor of fruit foraging efficiency—lemurs forage more rapidly on smaller fruits relative to larger fruits.

Introduction

Variation in foraging on fruits by animals can be attributed to many aspects of both animal sensory phenotypes and fruit traits (Wheelwright 1985; Hiramatsu et al. 2009; Melin et al. 2009; Lomáscolo and Schaefer 2010). The evolution of phenotypes that influence the senses of frugivorous animals, such as colour vision and olfactory ability, has been linked to increasing foraging efficiency on ripe fruits (Melin et al. 2009). Similarly, variation in fruit traits has been attributed, at least partially, to its role in attracting animals that provide important seed dispersal services (Howe and Smallwood 1982; Janson 1983; Wheelwright and Janson 1985; Lomáscolo et al. 2010; Valenta et al. 2013). However, the extent of the relationship between fruiting plants and the animals that disperse their seeds remains poorly understood.

The question of whether plants have evolved specialized fruit signals, such as colour and odour, to attract dispersal agents is contentious, because fruits are commonly dispersed by multiple taxa with diverse sensory phenotypes and phylogenetic backgrounds (Herrera 1987; 1992; Fischer and Chapman 1993). Thus, despite the intuitive appeal of the idea that frugivores may be driving the evolution of fruit traits, specialized fruit traits encouraging a restricted set of seed

✉ Kim Valenta
valentakim@gmail.com

1 Department of Anthropology, 19 Russell Street, University of Toronto, Toronto, ON M5S 2S2, Canada

2 Department of Anthropology, McGill University, 855 Sherbrooke Street West, Montreal, QC H3A 2 T7, Canada

3 Dalla Lana School of Public Health, University of Toronto, 155 College Street, Toronto, ON M5T 3M7, Canada

4 Department of Paleontology, University of Antananarivo, Antananarivo, Madagascar, Africa

5 Leibniz Institute for Tropospheric Research, Permoserstraße 15, 04318 Leipzig, Germany

6 Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON M5S 3H6, Canada

7 Wildlife Conservation Society, Bronx, NY, USA

8 Department of Anthropology, Washington University, Campus Box 1114, One Brooking Drive, St. Louis, MO 63130, USA

dispersers may be selected against (Schaefer et al. 2007) or result in limited fruit diversification or fruit trait convergence (Wheelwright and Orians 1981; Whitney 2009). Some studies have found evidence of fruit trait signal convergence in species dispersed by multiple frugivorous guilds, which contradicts the notion that certain frugivore species are driving the evolution of fruit traits (Wheelwright and Janson 1985).

Despite evidence against tight fruit-frugivore co-evolutionary relationships, there is some empirical support for it. Several studies have discovered strong correlations between frugivore phenotypes and fruit traits, which implies that fruit signal trait convergence might result, at least partially, from frugivore preferences for certain fruit traits (Chapman et al. 1992; Balcomb and Chapman 2003; Lomáscolo et al. 2008). Indeed, several fruit traits have been linked to frugivore phenotypes, including seed size and avian gape width (Wheelwright 1985; Mazer and Wheelwright 1993), fruit odour and nocturnal mammalian fruit choice (Lomáscolo et al. 2010; Valenta et al. 2013), and fruit colour conspicuity and avian and mammalian colour vision phenotypes (Wheelwright and Janson 1985; Lomáscolo and Schaefer 2010).

Results are also mixed with regard to whether fruits drive the evolution of sensory phenotypes in frugivores. While there is compelling evidence to suggest that variation in fruit traits has been responsible for variation in frugivore sensory phenotypes, such as polymorphic colour vision (Melin et al. 2009), there is also evidence to suggest that the evolutionary drivers of animal sensory modalities lie elsewhere, including fallback foods (Dominy and Lucas 2001) and nutritionally critical foods (Valenta and Melin 2012). Measuring the chromaticity of young leaves and fruit consumed by trichromatic primates Dominy and Lucas (2001) found that while trichromacy was necessary for distinguishing young leaves, dichromacy was sufficient for distinguishing ripe fruits, implying that foraging on leaves, and not fruit, drives the evolution of primate colour vision. Another study of platyrrhine feeding preferences found evidence for the importance of limiting nutritional resources, specifically protein, in the evolution of primate visual phenotypes (Valenta and Melin 2012).

Since both animal sensory phenotypes and fruit traits are diverse, a means to elucidate the evolutionary importance of fruit-frugivore interactions would involve examining the role of fruit traits on taxon-specific foraging behaviour. Previous studies of fruit traits and frugivore foraging have discovered correlations between frugivore sensory phenotypes and foraging behaviour and fruit traits. Olfactory-driven foragers, from taxa as diverse as bats, nocturnal primates, and coatis, have been found to respond to variation in fruit odour (Korine et al. 2000; Sanchez et al. 2006; Hodgkison et al. 2007; Hirsch 2010; Valenta et al. 2013), and several studies have documented the importance of fruit colour in diurnal bird and mammal foraging decisions (Debussche and Isenmann 1989; Schaefer

et al. 2006, 2007; Melin et al. 2009). However, to our knowledge, no study has related foraging behaviour in the wild to quantitative measures of fruit hardness, colour, and odour. Given the multiple sensory cues available to foraging animals, a multi-sensory approach is an important means of elucidating the interaction between animal behaviour, fruit signals and cues, and foraging outcomes (Dominy 2004).

Here, we quantify the extent to which fruit traits affect brown lemur (*Eulemur fulvus*) foraging behaviour and efficiency. Strepsirrhine primates (lemurs, lorises, and galagos) provide an excellent means of measuring the interplay between fruit traits, sensory systems, and animal foraging behaviour because their olfactory and visual systems are intermediate to most mammals and other primates (Rushmore et al. 2012). Relative to other mammals, primate sensory evolution is generally marked by decreased reliance on olfaction and increased visual adaptations (Gilad et al. 2004). However, relative to other primates, strepsirrhines retain extensive neuroanatomical structures associated with olfactory discrimination, including moist rhinaria, functioning vomeronasal organs, large olfactory bulbs (Barton et al. 1995), and lower visual acuity (Kirk and Kay 2004). Brown lemurs, specifically, are a particularly interesting taxon to measure the interplay between fruit signals and sensory phenotypes because they are highly frugivorous and their capacity for colour vision can be accurately modelled based on known peak cone spectral sensitivities and optical morphology (Jacobs and Deegan 1993; Peichl et al. 2001). A previous study of seed dispersal by *E. fulvus* found that most seeds ingested by brown lemurs were defecated intact and had higher germination rates relative to seeds that were not ingested and defecated by brown lemurs (Sato 2012).

We test the hypotheses that variation in fruit traits drives variation in foraging behaviour and foraging efficiency through our study of brown lemurs. We test these hypotheses using both bivariate associations between fruit traits and lemur foraging behaviour and efficiency. We also examine the effect of each fruit trait on foraging behaviour and efficiency while controlling for the effect of all other fruit traits using multivariate analyses. Given that the evolution of animal foraging behaviours and phenotypes depends critically on the need to detect and select food items (Dominy 2004), we predict that there will be significant positive relationships between (1) tactile foraging and fruit hardness, (2) visual foraging and visual conspicuity, and (3) olfactory foraging and overall fruit odour. We additionally hypothesize that variation in fruit traits will drive variation in foraging efficiency and quantify which fruit signals have the greatest effect on foraging efficiency. We predict that foraging efficiency will be higher for (1) fruits with a higher ratio of ripe/unripe puncture resistance, (2) fruits that are more visually conspicuous, and (3) fruits with stronger smell signals.

Methods

Behavioural data collection

Data were collected adjacent to Ampijoroa forestry station in the tropical dry forest in Ankarafantsika National Park, north-western Madagascar (ANP—15″ 59′–16° 22″ S, 47″ 56′–47″ 12′ E). Three habituated groups of brown lemurs with a total of 29 individuals were observed for 12 months (January–December 2012). Brown lemurs are highly frugivorous, sexually monomorphic, medium-sized cathemeral lemurs averaging 1.7–2.1 kg (Mittermeier et al. 2010). Groups A and B were habituated during a pilot season from July to December 2011, while group C was habituated in May of 2012. Group sizes ranged from five to ten individuals at any one time, with several individuals disappearing from groups and new individuals appearing (see Valenta 2014 for a description of group compositions).

Three observers conducted 5-min focal animal follows in the wet season (January to May 2012) and 10-min focal animal follows (Altmann 1974) in the dry season (May to December 2012) on all animals in the three groups. Focal animal sampling was conducted between 0500 and 1830 hours 4 days each week, for a total of 1366 contact hours. When the focal animal was not visible for greater than 10 % of a sample, the data were discarded. Behavioural data were recorded using a dictaphone and then transcribed.

Observers recorded broad behavioural categories (e.g. travelling, resting), and each time the focal animal fed on fruit, observers recorded detailed foraging behaviour, as well as the food item and part consumed, e.g. ripe fruit, unripe fruit, and leaf (see Valenta 2014 for a description of the behaviours). Each month, KV conducted inter-observer reliability tests which consisted of comparing 5-min long observer records taken simultaneously and in all cases inter-observer reliability remained >95 %.

Fruit collection

Each time a focal animal consumed fruit, fruits were collected directly from the tree after focal animals left it, and we attempted to collect fruits that appeared to the human observer to match the traits used by *E. fulvus* in their selection. While definitively identifying fruit ripeness is extremely difficult, observers attempted to collect fruits of the same size and colour as those on which animals were foraging. This may introduce error, as animals may feed on unripe fruits, especially in times of fruit scarcity (Worman and Chapman 2005; Masette et al. 2015). In all cases, to try to avoid introducing error, fruits were collected at a time when brown lemurs were defecating seeds of that species intact, and after colour and odour sampling, fruits were opened and seeds examined visually to further help discern ripeness based on seed morphology. Where

possible, fruits were identified to species and analysed. Mature leaves of the same species were also collected for trait analysis. Plants were identified to genus and species where possible using a published flora (Schatz 2001) and an unpublished photographic database of the plants of Ankarafantsika National Park (H. Sato, pers comm). In cases where it was not possible to identify plants to the genus level, they were identified either by their local Malagasy name or as an unknown species with a given number.

Measuring fruit exocarp hardness

Puncture resistance of ripe and unripe fruits was measured using a modified force gauge (Shimpo MF 50)—the edge of the force gauge was inserted at a 90° angle into individual fruits until the exocarp was punctured. The mean force in pounds (to a maximum of 50 lbs) required to puncture fruit exocarp was recorded for between five and ten ripe and unripe fruits of each species. The fruit hardness results thus represented the weight (in pounds) of force required for a probe measuring 4 mm in diameter to puncture fruit exocarp, and hardness values were calculated to represent kilograms of force per square millimetre after (Kinzey and Norconk 1990) by dividing raw values by 12.56×2.20462. In cases where ripe or unripe fruits exceeded a puncture resistance of 50 lbs (the maximum sampling ability of the force gauge), species were scored as 50 lbs. Ripe fruit mass was recorded using a digital scale (KERN 440 35A; precision ± 0.1 g; Table 1).

Capturing and analysing volatile organic compounds

To quantify fruit odour, ripe fruits of 55 species were collected, returned to the laboratory, and placed inside plastic sampling bags (Reynolds large oven bags). The atmosphere within each bag was sampled using a vacuum pump (Gilian 5000, Sensidyne), which pulled air through the sample bag (1 L/min, 240 min) and into two odorant-absorbent filters (Amberlite XAD-2, 400–200 mg, Sigma-Aldrich). Contamination of the sampling enclosure with ambient volatile organic compounds (VOCs) was minimized by passing incoming air through a container of activated carbon. Trapped VOCs were analysed using the procedures and instrumentation reported in Valenta et al. (2013). All VOC sums were divided by the total surface area of sampled fruits to control for fruit size.

Fruit colour sampling

Reflectance spectra of ripe fruits (targets) and upper leaf surfaces (backgrounds) were measured relative to a Spectralon white reflectance standard (Labsphere) on-site in Madagascar using a Jaz portable spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics Inc.) emitting a D-65 light source. The fruit scanning angle was fixed at 45°, and external light was

Table 1 List of all fruits eaten by three habituated groups of brown lemurs (*Eulemur fulvus*) in Ankarafantsika National Park, north-western Madagascar

Species	Average fruit mass (grams)	SD Fruit mass	Range Fruit mass	Main ripe fruit puncture resistance (kg/mm²)	Puncture resistance variance	N fruit sampled
Antidesma petiolare	0.07	0.01	0.06–0.08	0.24	0.01	5
Asterotrichilia asterotricha	9.92	0.74	8.97–10.83	0.61	0.03	5
Asterotrichilia merina	3.71	0.21	3.46–3.98	1.89	0.01	5
Badouinia fluggeiformis	0.84	0.09	0.72–0.95	2.63	0.35	5
Berchemia discolour	2.23	0.22	2.02–2.53	1.25	0.03	5
UK spp 1	0.24	0.06	0.10–0.27	0.04	0.003	10
Bridelia pervilleana	0.19	0.01	0.18–0.22	0.13	0.002	10
Croton spp	0.06	0.01	0.05–0.08	0.16	0.002	10
Fofona ratsy	26.64	13.58	16.82–48.89	2.99	1.87	5
Gaertnera spp	0.28	0.09	0.16–0.44	0.67	0.004	10
Garcinia arenicola	4.96	0.58	4.30–5.66	0.60	0.008	5
Gardenia rutenbergiana	15.02	6.29	10.9–26	0.13	0.002	5
Grewia madagascariensis	1.66	0.42	0.95–2.04	1.23	0.11	5
Grewia spp 1	1.28	0.21	0.97–1.48	8.78	0.000	5
Grewia triflora	0.387	0.05	0.34–0.46	1.26	0.10	10
UK spp 2	0.42	0.06	0.34–0.49	0.24	0.03	5
Asterotrichilia spp	15.10	0.85	13.94–16.03	1.57	0.06	5
Mapouria boinensis	0.61	0.13	0.39–0.70	0.06	0.002	8
Monanthotaxis valida	0.87	0.26	0.57–1.09	2.27	0.18	5
Olax madagascariensis	1.37	0.16	1.26–1.64	0.29	0.01	5
Rothmania renniformis	4.99	1.22	3.90–6.82	0.06	0.001	5
Sorindeia spp	6.87	0.77	6.16–8.37	0.35	0.001	6
Strychnos decussate	5.12	1.17	3.97–6.72	3.14	0.13	6
Strychnos madagascariensis	22.63	5.61	15.44–30.22	5.15	1.79	6
Strychnos myrtoides	0.68	0.12	0.48–0.76	0.16	0.0004	5
Terminalia tropophylla	0.13	0.03	0.09–0.18	1.62	0.01	5
Tricalysia perrieri	0.30	0.05	0.22–0.39	0.04	0.0006	10
UK Liana 1	0.56	0.18	0.30–0.80	0.47	0.02	5
UK Liana 3	0.32	0.07	0.23–0.41	0.002	0.00	10
UK Liana 4	0.78	0.42	0.50–1.64	0.48	0.002	6
Vitex beraviensis	1.76	0.45	1.2–2.4	1.84	0.07	5
Vitex perrieri	1.25	0.15	1.10–1.42	1.65	0.03	5
Vitex spp	2.43	0.50	1.61–2.90	0.90	0.08	5
Ximenia caffra	1.57	0.89	0.83–2.93	0.57	0.02	5
Ziziphus spp	6.54	1.75	4.32–8.59	1.16	0.12	5

Those included in behavioural analyses, along with average fruit mass, S.D. fruit mass, and range of fruit mass (grams), mean ripe fruit puncture resistance and variance (in kg/mm²) and number of fruits sampled per species

blocked using thick black fabric. For the representative irradiance spectrum, we used forest shade (Melin et al. 2012). The spectral sensitivity function for each photoreceptor type was calculated as per Valenta et al. (2013). Although rods may contribute to colour perception at dim light levels, the perceptual effects of this are not well understood, and as such, we omitted the contribution of rods for simplicity.

We judged the conspicuity of fruits by completing a just noticeable difference (JND) analysis for fruit against background leaves of the same species, where 1 JND represents the smallest difference that can theoretically be perceived (Osorio et al. 2004; Allen and Higham 2013). The JND model was parameterized after Matsumoto et al. (2014), including the relative cone proportions—which are reported to be similar between *Eulemur* and New World monkeys (Martin and Grunert 1999; Peichl et al. 2001). The chromatic and luminance conspicuity of food items was modelled as a ratio of the quantum catch of photons incident on the

retina by different cone types following established methods (Sumner and Mollon 2000; Osorio et al. 2004; Hiramatsu et al. 2008), using a dichromatic visual model based on the long-wavelength-sensitive (L) photopigments (λ_{max} 558 nm) and short-wavelength-sensitive (S) photopigments (λ_{max} 413 nm) possessed by *Eulemur* (Jacobs and Deegan 1993; Veilleux and Bolnick 2009; Carvalho et al. 2012). We additionally varied the estimated quantum flux (q) of photons incident on the cones (q) according to the forest shade irradiance condition: $q_{forest\ shade} = 10^4$ following Osorio et al. (2004).

Statistical analyses

To test the association amongst fruit traits, we compared fruit traits to each other using Pearson correlation. To measure the effect of fruit traits on fruit foraging behaviour and fruit foraging efficiency (rate of fruit ingestion), we first identified all fruit species for which complete morphological data (chromatic JND, luminance JND, VOC sum, hardness, size, and mass) were available and for which there were a minimum of ten associated behavioural records ($N=34$). We then calculated a hardness index (HI) comprised of the puncture resistance of a given unripe fruit/ripe fruits of that species. We then created a dataset of fruit foraging attempts (FFA, $N=607$), which are defined as a period during which an animal was foraging on a particular fruit species (e.g. time in one fruiting tree). For each FFA, we calculated a sniffing index (SI), a visual inspection index (VI) and a touching index (TI). These indices were defined as the time per foraging attempt spent using a certain sensory modality (e.g. sniffing)/the total amount of time in that FFA. We additionally calculated an acceptance index (AI) which was defined as the number of fruits eaten/the number of fruits investigated using each sensory modality (touch, visual inspection, sniffing) per FFA (Table 2). FFA measures per species were averaged giving each FFA an equal weight, irrespective of FFA duration, yielding a species-level dataset. Subsequent analyses were performed on the species-level dataset.

To test the association between fruit foraging behaviour and fruit traits, we ran four bivariate weighted linear regression models (LMs) at the species level ($N=34$): SI was compared to VOC sum, TI was compared to HI, and VI was compared to chromatic and luminance JND values. Weights corresponded to the number of FFAs per fruit species. During exploratory analyses, a confounding effect of fruit size as proxied by mass was detected. As such, we additionally ran a multivariate linear regression model that included all four predictor variables (chromatic JND, luminance JND, VOC sum, hardness index), with the addition of fruit mass. Fruit mass was found to be a good proxy of fruit volume. To determine this, we measured fruit size in three dimensions and then calculated fruit volume based on the calculation for an ellipse. We found that fruit volume and mass were highly correlated

($r=0.98$, $p<0.001$) and since error is more likely to be introduced by measuring the three dimensions, we used fruit mass as a proxy of size. Next, to test the association between the AI and fruit traits, we conducted five bivariate LMs with an intercept term. As above, we also ran a multivariate LM with all four predictor variables and mass to adjust for the potential confounding effects of other fruit traits. For all multivariate models, we assessed multicollinearity using the variance inflation factor. All variance inflation factors were beneath the generally proposed cut-off value of 10 (Neter et al. 1996). Specifically, the variance inflation factors were 3.1 for chromatic JND, 2.5 for luminance JND, 1.2 for VOC sum, 1.5 for hardness index and 1.2 for fruit mass. All reported p values were two-tailed, and statistical analyses were conducted in the R statistical software package (version 3.0.2).

Results

Of the fruit traits measured, two traits were correlated: Chromaticity was positively correlated with luminance ($r=0.46$, $p=0.01$), and chromaticity was negatively correlated with VOC sum ($r=-0.38$, $p=0.02$), i.e. fruits emitting a strong smell signal tended to be less chromatically conspicuous (Table 3).

Lemurs spent significantly more time visually inspecting visually conspicuous fruits (fruits with a JND value>1, chromatic conspicuity $F=10.8$, $df=1$, 32, $p=0.002$, Fig. 1b; luminance conspicuity $F=4.8$, $df=1$, 32, $p=0.03$, Fig. 1c), than less conspicuous fruits. They did not spend more time touching fruits with higher ripe/unripe hardness ratios ($F=0.8$, $df=1$, 32, $p=0.38$, Fig. 1d). Contrary to our prediction, lemurs did not spend more time sniffing fruits with high VOC sums ($F=0.36$, $df=1$, 32, $p=0.55$, Fig. 1a).

The hypothesis that variation in fruit traits drives variation in foraging behaviour is not supported by the multivariate model which measures the effect of each fruit morphological trait while holding all other traits constant. Here, the significant effect of chromatic conspicuity on visual foraging disappears ($F=0.03$, $df=1$, 28, $p=0.87$) as does the luminance conspicuity effect ($F=1.4$, $df=1$, 28, $p=0.24$). The effect of the hardness index remained non-significant ($F=0.45$, $df=1$, 28, $p=0.45$). Interestingly, the negative effect of VOCs on the sniffing index is amplified and approaches significance, when controlling for other fruit morphological relationships ($F=3.8$, $df=1$, 28, $p=0.06$).

In bivariate models, the hypothesis that fruit traits drive foraging efficiency is only partially supported. The acceptance index of ripe fruits did not vary as a function of chromatic conspicuity ($F=2.7$, $df=1$, 32, $p=0.11$, Fig. 2b). Similarly, luminance conspicuity did not affect foraging efficiency ($F=0.9$, $df=1$, 32, $p=0.35$, Fig. 2c). There was a significant negative relationship between the acceptance index of fruits

Table 2 List of all fruits eaten by three habituated groups of brown lemurs (*Eulemur fulvus*) in Ankarafantsika National Park, north-western Madagascar

Species	FFA records	Total seconds	Sniff index	Visual inspection index	Touching index	VOC sum	Luminance JND	Chromatic JND	Hardness index	N fruit sampled
Antidesma petiolare	5	227	0.48	0.48	0.04	34.34	16.25	0.86	1.42	5
Asterotrichilia asterotricha	44	2327	0.53	0.29	0.17	3.97	3.17	1.43	0.16	5
Asterotrichilia merina	16	736	0.38	0.5	0.12	1.78	34.06	2.79	0.65	5
Badouinia fluggeiformis	3	105	0.09	0.37	0.54	5.26	19.88	2.35	0.3	5
Berchemia discolor	24	934	0.44	0.34	0.22	4.51	23.99	1	0.64	5
UK spp 1	3	129	0.49	0.5	0.02	1.28	21.62	1.72	0.13	10
Bridelia pervilleana	26	843	0.44	0.49	0.07	2.75	23.63	1.46	0.07	10
Croton spp	14	1012	0.5	0.36	0.14	7.58	23.55	2.26	0.21	10
Fofona ratsy	13	352	0.54	0.35	0.04	13.95	23.16	0.63	1.15	5
Gaertnera spp	5	80	0.62	0.06	0.32	2.75	11.66	0.32	0.6	10
Garcinia arenicola	10	206	0.65	0.14	0.21	1.28	14.9	1.79	0.7	5
Gardenia rutenbergiana	78	2247	0.21	0.62	0.15	4.83	17.19	1.79	0.03	5
Grewia madagascariensis	3	89	0.66	0.18	0.17	0.22	49.08	0.63	0.8	5
Grewia spp 1	2	57	0.36	0.57	0.07	10.3	0.01	0.02	1	5
Grewia triflora	151	13875	0.61	0.32	0.07	5.76	8.66	0.07	0.91	10
UK spp 2	4	44	0.38	0.53	0.1	9.73	10.06	3.43	0.43	5
Asterotrichilia spp	5	255	0.29	0.54	0.16	3.97	24.56	2.54	0.53	5
Mapouria boinensis	10	554	0.39	0.37	0.24	15.86	19.37	1.09	0.05	8
Monanthotaxis valida	47	3056	0.24	0.51	0.25	10.93	42.23	3.65	0.21	5
Olax madagascariensis	13	547	0.37	0.46	0.17	45.91	6.19	0.06	0.22	5
Rothmania renniformis	13	339	0.31	0.54	0.15	24.18	14.64	1.09	0.01	5
Sorindeia spp	11	426	0.65	0.2	0.15	17.14	5.7	0.8	0.42	6
Strychnos decussate	2	99	0.25	0.14	0.61	53.44	23.73	0.15	0.37	5
Strychnos madagascariensis	9	315	0.44	0.27	0.29	3.84	33.04	3.04	0.59	6
Strychnos myrtoides	2	31	0.28	0.7	0.02	5.86	8.97	0.08	0.17	5
Terminalia tropophylla	44	2367	0.44	0.34	0.22	34.34	1.52	0.47	0.18	5
Tricalysia perrieri	10	1162	0.39	0.57	0.05	15.75	17.53	1.5	0.05	10
UK Liana 1	3	162	0.48	0.52	0	9.35	2.71	0.38	0.7	5
UK Liana 3	9	703	0.25	0.67	0.08	2.81	13.26	2.99	0	10
UK Liana 4	1	33	0.33	0.03	0.64	3.08	11.68	1.44	0.93	6
Vitex beraviensis	18	1626	0.47	0.43	0.1	2.26	10.03	1.6	0.49	5
Vitex perrieri	4	223	0.26	0.4	0.34	4.83	4.26	1.43	0.71	5
Vitex spp	1	193	0.16	0.81	0.03	1.28	13.13	1.64	0.45	5
Ximenia caffra	1	43	0.44	0.35	0.21	1.54	19.73	1.58	1.65	5
Ziziphus spp	3	268	0.24	0.55	0.19	0.25	1.65	0.35	20.49	5

Those included in behavioural analyses, along with number of fruit foraging attempts, time in seconds spent foraging, behavioural indices, fruit traits and number of fruits sampled per species

FFA fruit foraging attempts, *VOC* volatile organic compounds, *JND* just noticeable difference

relative to the hardness index ($F=3.98$, $df=1$, 32, $p=0.05$, Fig. 2d)—fruits with less variation in unripe to ripe puncture resistance were eaten more rapidly. There was no relationship between the acceptance index and VOC ($F=2.4$, $df=1$, 32, $p=0.13$, Fig. 2a). A significant negative relationship between acceptance index and fruit mass was noted ($F=9.4$, $df=1$, 32, $p=0.004$, Fig. 2e) and justified our inclusion of fruit size in the multivariate analysis of the acceptance index.

The results of the multivariate LMs indicate that only fruit size contributes meaningfully to brown lemur foraging efficiency. There is a negative relationship between the acceptance index and fruit size, such that brown lemurs consumed small fruits at a higher rate relative to overall foraging attempts ($F=4.3$, $df=1$, 28, $p=0.05$). There is no relationship between visual cues or the hardness index, foraging efficiency or VOC signal (chromatic JND $F=0.01$, $df=1$, 28, $p=0.90$; luminance

Table 3 Correlations between fruit traits using Pearson correlation, showing a positive correlation between fruit chromatic and luminance conspicuity, and a negative correlation between fruit chromatic conspicuity and VOC

	VOC sum	Luminance JND	Chromatic JND	Hardness index	Average mass (g)
VOC Sum	1				
Luminance JND	−0.14 (0.41)	1			
Chromatic JND	−0.38 (0.02)	0.46 (0.01)	1		
Hardness Index	0.10 (0.57)	−0.00 (0.99)	0.22 (0.19)	1	
Average mass (g)	−0.06 (0.71)	0.18 (0.29)	0.12 (0.48)	−0.09 (0.62)	1

p values are in brackets

JND $F=0.02$, $df=1, 28$, $p=0.89$; hardness index $F=1.2$, $df=1, 28$, $p=0.27$, Table 4).

Discussion

Fruit traits do not strongly influence lemur foraging behaviour or efficiency in the predicted directions. When controlling for the effect of all fruit traits, there was no association between the time lemurs spent visually inspecting fruits and larger chromatic or luminance conspicuity. Furthermore, contrary to our prediction, neither increased chromatic nor increased luminance conspicuity

increased acceptance index, suggesting that foraging efficiency is not responsive to increased visual conspicuity. Lemurs did not consume visually conspicuous fruits at a faster rate than visually inconspicuous fruits. A potential explanation for these results is that conspicuous chromaticity in fruits is negatively related to how odiferous a fruit is, as noted in other studies (Melin et al. 2009).

With regard to the effect of tactile signals on foraging behaviour, the intraspecific variation between ripe and unripe exocarp puncture resistance did not affect lemur's use of tactile foraging behaviours. Here again, the increased tactile signal between ripe and unripe fruits available to foraging lemurs did not increase their foraging efficiency.

Fig. 1 Scatterplot showing the effect of fruit traits on the foraging behaviours of three habituated groups of brown lemurs (*Eulemur fulvus*) in Ankarafantsika National Park, north-western Madagascar. **a** VOC sum and sniffing index ($y=0.45–0.001\times$). **b** Chromatic JND values and visual inspection index ($y=0.33+0.06\times$). **c** Luminance JND values and visual inspection index ($y=0.35+0.004\times$). **d** Hardness index and touching index ($y=0.13+0.02\times$). The size of the circle is proportional to the number of FFAs for each fruit with larger symbols meaning more FFAs and more stable estimates
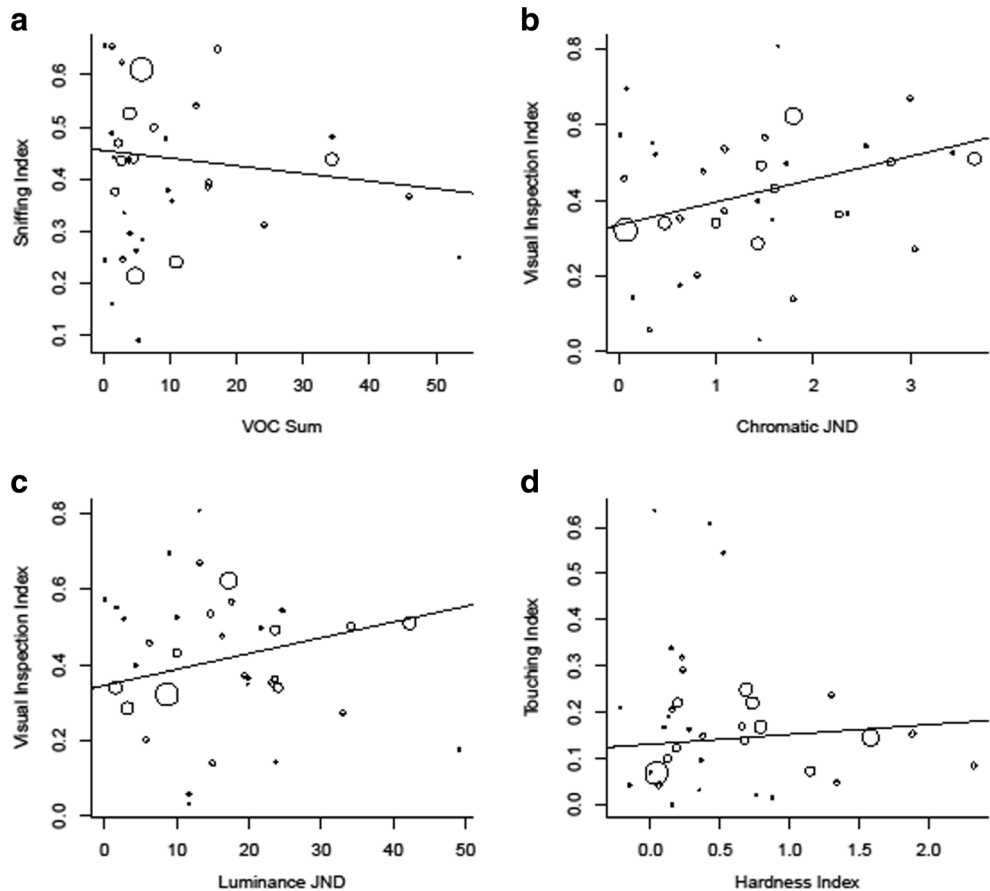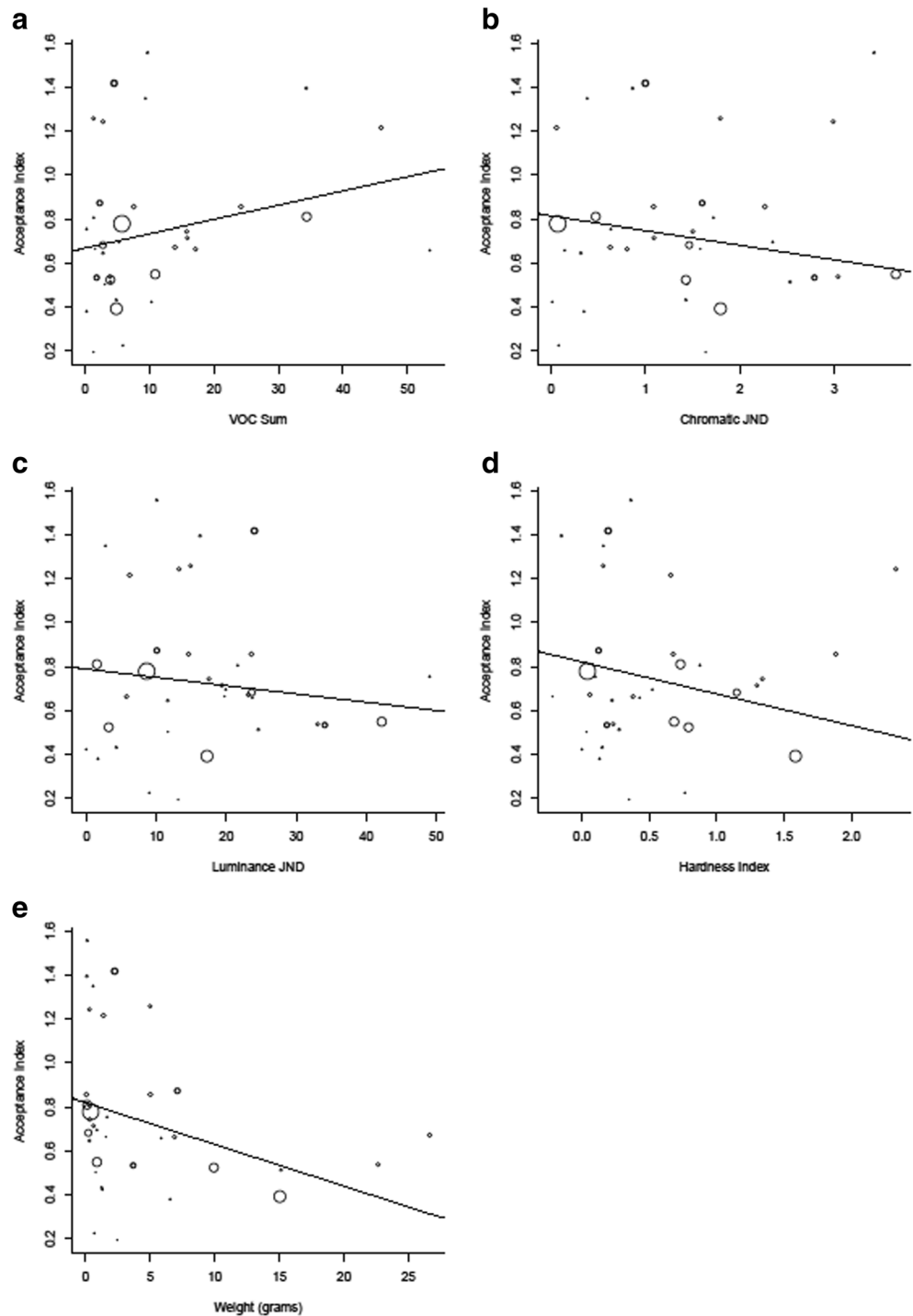
Fig. 2 Scatterplot showing the effect of fruit traits on lemur fruit foraging efficiency (acceptance index) of three habituated groups of brown lemurs (*Eulemur fulvus*) in Ankarafantsika National Park, north-western Madagascar. **a** VOC sum ($y=0.67+0.006\times$). **b** Chromatic JND values ($y=0.81-0.07\times$). **c** Luminance JND values ($y=0.79-0.004\times$). **d** Hardness index ($y=0.82-0.14\times$). **e** Fruit mass ($y=0.82-0.02\times$). The *size of the circle* is proportional to the number of FFAs for each fruit with larger symbols meaning more FFAs and more stable estimates

Lemur olfactory foraging behaviour was marginally associated with fruit VOC sum, but not in the direction predicted. Fruit VOC sum was not associated with foraging efficiency. In multivariate analyses, higher VOC showed a marginally significant negative association with the sniffing index, and this may result from two possibilities. Firstly, it is possible that the need to sniff fruits with strong smell signals for shorter periods of time is precisely because there is greater olfactory

information available. Thus, lemurs can reduce their time spent sniffing high VOC fruits relative to low VOC fruits. This is supported by the positive trend between fruit foraging efficiency and smell signals—lemurs tend to eat fruits at a higher rate relative to fruit inspection when those fruits have stronger smell signals. Alternately, it is likely that overall smell signal is not the only predictor of olfactory foraging behaviour. Olfactory receptor gene repertoires have not yet

Table 4 Results of unadjusted (bivariate) and adjusted (multivariate) linear models

	Acceptance index		Visualization index		Sniffing index		Touching index	
	Unadjusted[a]	Adjusted[a]	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
VOC sum	$F=2.36$ Positive $p=0.134$	$F=1.30$ Positive $p=0.263$	$F=0.10$ Negative $p=0.755$	$F=0.13$ Negative $p=0.724$	$F=0.36$ Negative $p=0.554$	$F=3.85$ Negative $p=0.059$	$F=2.82$ Positive $p=0.103$	$F=6.82$ Positive $p=0.014$
Luminance JND	$F=0.89$ Negative $p=0.351$	$F=0.02$ Negative $p=0.886$	$F=4.80$ Positive $p=0.036$	$F=1.44$ Positive $p=0.239$	$F=9.58$ Negative $p=0.004$	$F=1.55$ Negative $p=0.223$	$F=3.06$ Positive $p=0.090$	$F=0.03$ Negative $p=0.869$
Chromatic JND	$F=2.68$ Positive $p=0.111$	$F=0.01$ Negative $p=0.904$	$F=10.82$ Positive $p=0.002$	$F=0.03$ Positive $p=0.867$	$F=27.49$ Negative $p=0.000$	$F=4.21$ Negative $p=0.049$	$F=6.87$ Positive $p=0.013$	$F=4.81$ Positive $p=0.036$
Hardness index	$F=3.98$ Negative $p=0.054$	$F=1.24$ Negative $p=0.275$	$F=30.51$ Positive $p=0.000$	$F=18.44$ Positive $p=0.000$	$F=35.14$ Negative $p=0.000$	$F=18.34$ Negative $p=0.000$	$F=0.78$ Positive $p=0.384$	$F=0.58$ Negative $p=0.452$
Average mass (g)	$F=9.40$ Negative $p=0.004$	$F=4.30$ Negative $p=0.047$	$F=2.50$ Positive $p=0.124$	$F=0.01$ Positive $p=0.921$	$F=3.96$ Negative $p=0.055$	$F=1.03$ Negative $p=0.319$	$F=0.10$ Positive $p=0.754$	$F=0.10$ Positive $p=0.759$

[a] F statistics for unadjusted models have 1/33 DFs; for adjusted models, there are 1/29 DFs

been sequenced for this genus, and brown lemur's phenotypic sensitivity to specific VOCs is unknown. Additionally, physiological evidence supporting a positive correlation between the number of functional olfactory receptor genes and olfactory performance is largely lacking (Shepherd 2004; Laska et al. 2006). While higher overall VOC signals may result in lower time spent sniffing, it is possible that brown lemurs, like other olfactory-driven foragers, are responsive to VOCs that are present even in trace amounts (Linn et al. 2004; Hodgkison et al. 2007). Thus, rather than identifying the overall VOC signal of fruits, it would be beneficial to additionally identify brown lemur sensitivity to certain VOCs, in addition to identifying their presence at critical thresholds in fruits (Valenta et al. 2013).

The fruit morphological characteristic with the strongest effect on fruit foraging efficiency was fruit size. After controlling for all fruit morphological traits, fruit size alone was the only significant predictor of foraging efficiency—brown lemurs consumed small fruits at a higher rate relative to overall foraging attempts than larger fruits. To understand the selective forces underlying the relationship between foraging efficiency and fruit size, it will be necessary to evaluate how nutrition of the fruits varies as a function of size and the costs of foraging on fruits of different sizes, as animals are attempting to maximize nutritional gains relative to cost, not simply maximize intake (Ganzhorn 2002; Rothman et al. 2012).

Overall, we found that fruit traits were not associated with foraging behaviour, which is counter to what would be expected by optimal foraging theory (Stephens and Krebs 1986). In terms of foraging efficiency, the only significant predictor was fruit size—lemurs were more efficient at foraging on smaller fruits. What our findings suggest with respect to optimal foraging theory is that examining a single trait or a suite of traits may be insufficient in explaining the foraging choices of animals. A more holistic approach using a multivariate statistical technique will more likely reveal the optimal decision rules used by a species in a specific location, at a specific time and with a specific set of foraging options. The geometric framework approach offers one such approach but would need to be expanded to include other traits animals could select such as odour (Raubenheimer et al. 2009). Furthermore, future research should clarify the degree of fruit-frugivore phenotypes across frugivorous guilds and floral assemblages. For example, comparing the fruit signals available to routinely trichromatic primates in continental Africa and Asia to fruit signals available to lemurs in Madagascar would be an interesting way of further elucidating the extent to which dietary fruit shapes frugivore sensory phenotypes.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study complies with all national and regional laws dealing with ethics and animal welfare in both Madagascar and Canada (University of Toronto (Animal Care Protocol #20009112).

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