

Fruit Ripening Signals and Cues in a Madagascan Dry Forest: Haptic Indicators Reliably Indicate Fruit Ripeness to Dichromatic Lemurs

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Abstract Fruit ripeness can be indicated through changes in chromaticity, luminance, odor, hardness, and size to attract seed dispersing animals. We quantified these attributes for both ripe and unripe fruits of 31 lemur-dispersed plant species in Ankarafantsika National Park, a tropical dry forest in northwestern Madagascar. We used spectroscopy, gas-chromatography mass-spectrometry, and a modified force gauge to quantify chromaticity, luminance, odor, and hardness. We compared these traits between unripe and ripe fruits of each species to determine which

traits reliably indicate fruit ripeness across species. Overall, ripe fruits were significantly heavier and softer than unripe fruits. Ripe fruits were not more chromatically-conspicuous or odiferous relative to unripe fruits, nor were ripe fruits more conspicuous in the luminance channel. Contrary to expectation, our findings indicate that, in this particular system, plant-lemur interactions may be strongly mediated by haptic traits, such as fruit hardness, which are consistent and reliable indicators of fruit ripeness. Despite the potential importance of haptic indicators of fruit ripeness, they are underrepresented in the literature on sensory ecology.

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Introduction

Most plant reproduction requires animals for seed dispersal or pollination, particularly in the tropics (Schaefer et al. 2007). Plant cues and signals are critical to ripe fruit detection and selection by animals, and thus seed dispersal (Linn et al. 2004; Korine and Kalko 2005; Hirsch 2010; Valido et al. 2011). Ripe fruit signals refer to traits that are actively selected for and maintained because of their ability to reliably convey information to dispersers (Schaefer and Braun 2009). In contrast, ripe fruit cues are characteristics that are better described as exaptations—informative traits that evolved without selective pressure from dispersers that nonetheless convey reliable information to dispersers (Otte 1974). Both signals and cues refer to traits that provide information to animals to help guide foraging decisions. In

the tropics, where at least 50 %, and often more than 75 %, of all tree species rely on birds and mammals for seed dispersal (Howe and Smallwood 1982), the question of which signals and cues are available to animals is especially relevant. Here, we quantify the signals and cues of fruits dispersed by dichromatic lemurs in a tropical dry forest in Madagascar.

Distinguishing between signals and cues in the context of seed dispersers and fruit traits is complex. While plant cues may result from myriad selective pressures unrelated to attracting dispersers, signaling theory predicts that effective, dependable signals must be costly to produce (Olson and Owens 1998). In the case of fruit traits that facilitate dispersal, identifying a fruit trait as a signal requires demonstrating that a given plant species incurs a cost (e.g., investment in pigments), to produce a signal (e.g., color). Further, it would have to be shown that the fruit trait corresponds to the sensory ability of the intended disperser (e.g., is visually detectable based on the disperser's visual phenotype) and is not primarily an adaptation arising from alternate selective pressures (e.g., protection from photoinhibition, cryptis from seed predators; Herrera 1985). Further, seed dispersal would have to exert sufficiently strong selective pressure to maintain the trait, in light of alternative selective pressures (Schupp 1993).

Despite the difficulty of distinguishing fruit signals and cues, the variation in how frugivorous taxa sensorily perceive fruits, and the variation in fruit traits among plant taxa have led many to hypothesize the existence of “dispersal syndromes” or “fruit syndromes” (Lomascolo et al. 2010). These syndromes are defined as co-occurring sets of fruits traits that correspond to the behavior, sensory adaptations, and morphology of different types of frugivores (Janson 1983; Gautier-Hion et al. 1985; Jordano 1995). One implication of the fruit syndrome hypothesis is that dispersers are the selective force behind fruit trait evolution (but see Fischer and Chapman 1993), though the fruit syndrome hypothesis does not explicitly identify the locus of trait selective pressure. Indeed, traits of a given plant species or fruit syndrome may be signals, cues, or a combination of signals and cues. In addition to the difficulty inherent in delineating signals and cues, whether or not plants have evolved disperser-specific signals is contentious because of the slow evolutionary rates of plants relative to animals, and the diversity of animals (i.e., diffuse selection) that disperse seeds of any given plant species (Fischer and Chapman 1993; Schaefer et al. 2007, Herrera 1985).

Plant signals are highly variable (Gautier-Hion et al. 1985) and comprise fruit chromaticity (Wheelwright and Janson 1985; Melin et al. 2008), brightness or luminance (Hiramatsu et al. 2008), odor (Sanchez et al. 2006),

hardness (Lambert et al. 2004), and size (Knight and Siegfried 1983). Earlier studies of the fruit traits associated with lemur fruit consumption and seed dispersal have focused on fruit and seed size, and have revealed high variation in lemur-dispersed fruit and seed size, likely resulting from the enormous variation in frugivorous lemur body size (Dew and Wright 1998; Ganzhorn et al. 1999; Lahann 2007). One study found that three species of large-bodied seed dispersing lemurs preferentially consumed large fruits (>10 mm in diameter), while studies of the physically diminutive lemurs of the family *Cheirogaleidae* found these lemurs consumed much smaller fruits, (median fruit length = 12.1 mm, Lahann 2007). In terms of other fruit traits, one early study found that three species of lemur preferred fruits that were green, brown, tan, purplish and red, though these colors were qualified according to human color vision phenotypes and categories (Dew and Wright 1998). Another study found that lemurs demonstrated no significant color or odor preferences based on qualitative human categories (Bollen 2007). Recent efforts to quantify the traits of lemur-dispersed fruits have found that chromaticity and overall volatile organic compound (VOC) production was correlated to fruit consumption and seed dispersal by two species of mouse lemurs (Valenta et al. 2013), while a study of *Eulemur fulvus* found that while fruit odor affected lemur foraging behavior, only fruit size affected lemur foraging efficiency, with increased foraging efficiency on smaller fruits (Valenta et al. 2015a). To our knowledge, no study to date has quantified and compared multivariate differences between ripe and unripe fruits in Madagascar forest.

Visual signals and cues, such as chromaticity and luminance, have long been identified as important indicators of fruit ripeness to seed dispersing animals (Allen 1879), with multiple color vision phenotypes. Amongst vertebrates color vision ranges from monochromacy to tetrachromacy (Osorio and Vorobyev 2008). Studies that quantify fruit chromaticity and luminance, and model it based on disperser visual phenotypes have demonstrated visually salient indicators of fruit ripeness for multiple taxa (Osorio et al. 2004; Schaefer et al. 2007), which suggests that seed dispersers can sometimes rely on optical indicators of ripeness in fruit selection.

Seed dispersing animals may also rely on olfaction to guide their foraging decisions (Dominy et al. 2001; Dominy 2004; Corlett 2011), and olfactory signals/cues have been shown to be used more often for fruit species that do not have clear visual indicators of ripening (Corlett 2011). While few studies have sampled olfactory receptor gene repertoires (Gilad et al. 2004; Dambroski et al. 2005; Rouquier and Giorgi 2007), evidence from experimental studies and broad neuroanatomical measures indicate considerable variation among vertebrates, which may result

from disparity in reliance on olfaction during foraging (Barton et al. 1995; Laska et al. 1999). Mature fruits contain nutrients, including fatty acids, amino acids, and carotenoids, which produce volatile metabolites often detectable by animals (Goff and Klee 2006). These “flavor volatiles” can function as reliable indicators of the nutritional quality of fruits to potential seed dispersers. Behaviorally, it has been shown that frugivorous primates smell fruit deliberately while foraging (Chapman and Chapman 1996; Dominy 2004; Valenta et al. 2015a), as do bats (Bianconi et al. 2012), and fruits vary in the emission, both qualitatively and quantitatively, of odors (Valenta et al. 2013; Nevo et al. 2015).

While understudied relative to other vertebrate senses, variation in haptic perception amongst vertebrates is likely high (Jacobson 1978), and studies of tactile sensitivity (Dominy et al. 2001) and behavioral studies have demonstrated animal reliance on haptic indicators of fruit ripeness during foraging (van Roosmalen 1985; Dominy et al. 2001). The tactile evaluation of foods during animal foraging can be obtained through contact with digits, teeth and/or the mouth (Dominy 2004; Corlett 2011). Thus, changes in fruit hardness and mass may also reliably indicate ripeness to seed dispersing mammals. Unlike color and odor, however, haptic indicators of fleshy fruit ripeness likely result from factors that are not directly related to animal signaling. Fruit hardness is a critical physical defense of unripe fruits (Kinzey and Norconk 1990; Lambert et al. 2004), and the softening of edible fruit tissue (mesocarp) is a developmental process based on the conversion of crystalline starch to soluble sugars (Paliyath et al. 2008). Softening of the exocarp characterizes the maturation of fruit and has been shown to progress more rapidly than changes in visual quality (Brady 1987). Ripe fruits generally weigh more than unripe fruits due to higher water content and this difference in mass may constitute an additional haptic indicator of fruit ripeness. Despite the unlikelihood that changes in fruit mass and hardness results from selective pressure from dispersers, these traits may nonetheless function as highly reliable indicators of fruit nutritional quality and ripeness (Dominy 2004). Because fruit mass and size are strongly positively correlated (Valenta et al. 2015b), fruit size/mass may also be thought of as a visual signal.

Here we evaluate traits associated with fruit-ripening in species dispersed by brown lemurs (*E. fulvus*) in Ankarafantsika, National Park, Madagascar. We compare quantitative measures of the chromaticity, luminance, odor, hardness, and mass of ripe and unripe fruits. We model chromaticity and luminance for the dichromatic phenotype and the peak spectral sensitivity of brown lemur (*E. fulvus*) photopigments, because brown lemurs are an important seed disperser in this forest, and because all fruits in the

present analysis were found to be dispersed by *E. fulvus*. However, this model can likely be accurately generalizable across dichromatic mammals. We predict: (1) puncture resistance of unripe fruit will be significantly higher than the puncture resistance of ripe fruit, i.e., ripe fruit will be softer than unripe fruit (Kinzey and Norconk 1990; Lambert et al. 2004); (2) ripe fruits will have higher mass than unripe fruits; (3) overall odor emissions of ripe fruits will be greater than the overall odor emissions of unripe fruits; (4) ripe fruits will be more conspicuous against a background of conspecific leaves than unripe fruits, and (5) ripe fruits will exhibit greater luminance and be more conspicuous against a background of conspecific leaves than unripe fruits. To rule out potential phylogenetic constraint on these fruit ripening signals, we first tested for the presence of any phylogenetic signal among traits using a species-level phylogeny.

Materials and Methods

Study Site and Species

Data were collected near the Ampijoroa field station in the tropical dry forest of Ankarafantsika National Park, northwestern Madagascar (UTM 38 K 694133 E/8195555 S), where there are three known avian frugivores, and five known mammalian seed dispersers, all of them lemurs (Langrand 1990; Mittermeier et al. 2010). All five large-bodied mammalian dispersers are lemurs and are either nocturnal or cathemeral dichromats (Jacobs and Deegan 1993; Tan and Li 1999; Mittermeier et al. 2010). We collected ripe and unripe fruits of species consumed by three habituated groups of brown lemurs over 12 months (January–December, 2012). We considered fruit ripe at the stage at which brown lemurs consumed the fruit and passed seeds intact in feces. After analysis, we further checked for ripeness based on the presence of viable seeds. Seeds were considered viable if they were fully formed and had the approximate mass of seeds known to germinate. We collected and analyzed mature leaves of these species. We identified plants to species where possible using a published generic tree flora (Schatz 2001) and an unpublished photographic database of the plants of Ankarafantsika National Park (Sato, pers. comm.). In cases where it was not possible to identify plants to the genus level (N = 11), they were identified by their Malagasy name, or classified as unknown species. Because the goal of the study is to identify fruit ripening signals in wild Malagasy plants, all non-endemic and human cultivated plant species (e.g., *Tamarindus indica*) were excluded. Additionally, sample design was not balanced because in some cases it was not possible to perform all analyses on both ripe and unripe fruits of a given species.

Hardness Sampling and Analysis

We measured the puncture resistance of ripe and unripe fruits of all species using a modified force gauge (Shimpo MF 50). The edge of the force gauge was inserted at a 90° angle into fruits until the exocarp was punctured. Fruit hardness is measured as the force (kg of force per mm²) required for a probe measuring 4 mm in diameter to puncture a fruit exocarp (Kinzey and Norconk 1990). This was recorded for between three and ten ripe and unripe fruits of each species. In cases where ripe or unripe fruits exceeded the maximum puncture resistance of the gauge, these fruits were scored as 22.68 kg or 50 lbs (the maximum force measurable by the gauge). Fruits were weighed using a digital scale and fruit weights were recorded to the nearest tenth of a gram.

Capturing and Analyzing Volatile Organic Compounds (VOCs)

To quantify fruit odor, we collected ripe and unripe fruits of 55 species. Fruits were returned to the laboratory within 4 h of collection and placed inside plastic sampling bags (Reynold's large oven bags). We sampled the atmosphere within each bag using a vacuum pump (Gilian 5000, Sensidyne) at a rate of 1 L/min for 240 min. Air was drawn through the bag into two odorant-absorbent filters (Amberlite XAD-2, 400–200 mg, Sigma-Aldrich). Contamination of the sampling enclosure with ambient VOCs was minimized by passing incoming air through activated carbon.

To analyze the trapped VOCs, we removed the XAD resin beds from their cartridges and manually shook them in 4 mL hexane (Sigma Aldrich) for 5 min. The two XAD beds were extracted separately. We analyzed extracts using an Agilent 7890A gas chromatograph interfaced with an Agilent 5975 inert mass spectrometer operating in electron ionization (EI) mode. All injection volumes were 1 µL and performed in the splitless mode with an inlet temperature of 250 °C. Separation was achieved using an Agilent DB-5 column (30 m × 0.25 mm × 0.25 µm) at a constant helium flow rate of 1 mL/min. The oven program consisted of an initial hold at 50 °C for 2 min, followed by a 10 °C/min ramp to 150 °C, and then a 30 °C/min ramp to 300 °C. The transfer line temperature was held at 300 °C. We monitored analytes in full scan mode using a selected mass range of 40–300 Da.

To control for variation in fruit number and surface area, we scaled the GC–MS chromatograms by the surface area of all fruits in the bag. To quantify surface area, we measured ripe and unripe fruits in three dimensions (height, width, and depth) using calipers, then calculated fruit surface area based on the mean dimensions using an equation for surface area for an ellipsoid.

We determined total VOC emission intensity for ripe and unripe samples of fruit species by extracting overall VOC peaks using MassLynx Software (V4.1, Waters) and summing the area under the surface-scaled GC–MS chromatograms using SigmaPlot (V9.01, Systat). We compared values for ripe and unripe fruits of the same species (N = 34 species).

Measuring and Analyzing Chromaticity and Luminance

We quantified fruit chromaticity and luminance for ripe and unripe fruits of 31 species. We measured the reflectance spectra of ripe fruits (targets) and upper leaf surfaces (backgrounds) relative to a Spectralon white reflectance standard (Labsphere) on-site in Madagascar with fresh material 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 blocked using thick black fabric (Valenta et al. 2013).

We modelled the chromaticity of fruits as the 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 SWS (λ_{\max} 413 nm) and MWS (λ_{\max} 545 nm) photopigments possessed by *E. fulvus* (Carvalho et al. 2012; Valenta et al. 2015c). The quantum catches of each photoreceptor (cone) type were calculated from the following equation:

$$Q_i = \int_{\min}^{\max} R(\lambda) \times I(\lambda) \times S_i(\lambda) \quad (1)$$

where Q_i represents the quantum catch of a photoreceptor i over the range of the primate visual spectrum, from 400 nm (min) to 700 nm (max), $R(\lambda)$ represents the reflectance spectrum, $I(\lambda)$ represents the irradiance spectrum, and $S_i(\lambda)$ is the spectral sensitivity function of the i -th photoreceptor (containing S and M photopigments). We used a measure for diurnal forest shade as the representative irradiance spectrum (Melin et al. 2012), and calculated the spectral sensitivity function for each photoreceptor type as per Hiramatsu et al. (2008), with one alteration. Because non-anthropoid mammals do not possess a *macula lutea*, the pre-receptor filter included only the effects of the lens, as opposed to the combined effects of the lens and macular pigment (Peichl et al. 2001). Although the rods may contribute to color perception at dim light levels, the perceptual effects of this are not well understood, accordingly we omitted the contribution of rods here for simplicity. The blue-yellow chromaticities of target and background objects can be represented and plotted as the relative quantum catches of the S cones to the M cones, S/M. We

defined the contrast for the blue-yellow cone channel as $\Delta_{ji} = \left| \ln(Q_i^f) - \ln(Q_i^b) \right|$ where Q^f and Q^b denote the quantum catches of the receptor i ($i = M$ and S) for fruit and background leaves (Hiramatsu et al. 2008).

Because the S cones do not contribute meaningfully to perception of luminance contrast, we estimated the relative luminance value of each object by dividing the quantum catch of the M cones by that of a hypothetical white surface that reflects 100 % of the illuminant. The luminance contrast ΔM was calculated as the contrast of M cones between fruit and background leaves, and modeled as $\Delta M_d = \Delta f_M$ after (Hiramatsu et al. 2008).

Phylogenetic Methods

We optimized blue-yellow chromatic contrast, luminance contrast, mean fruit weight, mean puncture resistance, and VOC emission onto a species-level phylogeny as continuous characters, using TNT version 1.1 (Goloboff et al. 2008). The model phylogeny was adapted from APG III and other classifications (Gadek et al. 1996; Backlund et al. 2000; Tokuoka and Tobe 2006; Goloboff et al. 2008; Bremer and Eriksson 2009; Chase and Reveal 2009; Nazar et al. 2012) and excluded unidentified morphospecies. We mapped the above characters onto the phylogeny for a maximally parsimonious arrangement, with an optimal range of values computed for each node. If any of these traits had a phylogenetic basis, the trait would cluster within groups of related taxa. Moreover, assuming maximum parsimony, a phylogenetically informative trait would exhibit minimal homoplasy. Thus, to evaluate optimized character distributions, we calculated consistency and retention indices for each trait (CI and RI, respectively). CI is a direct estimate of homoplasy (i.e., from 0 to 1, CI = 1 if there is none), while RI approximates how well the phylogenetic tree fits a character (i.e., from 0 to 1, RI = 1 if fit is perfect); i.e., if any of the measured traits are phylogenetically informative, both values should fall closer to 1 (Farris 1989). For a formal test of phylogenetic signal, we calculated Blomberg's K (Blomberg et al. 2003) and Pagel's λ (Pagel 1999) in R using the *phytools* package (Revell 2012) and compared results to a null model using the likelihood-ratio test.

Statistical Analyses

We used two modelling approaches: (1) Binomial mixed-effects regression to test which fruit traits may be used by Madagascan plants to signal ripeness to seed dispersing mammals. As there was no effect of phylogeny, phylogeny was not accounted for in the models; and (2) random forest models to test for the strength of association of the fruit

traits identified in the binomial regression with the dichotomous variable—ripeness (whether a species' traits were associated with ripe or unripe fruit). For summary purposes only, the differences in the traits between ripe and unripe fruits were examined using paired t tests.

(1) *Binomial mixed-effects regression*: We compared unripe and ripe fruit values for all five covariates (ripe vs. unripe fruit chromatic conspicuity, luminance conspicuity, puncture resistance, VOC and fruit mass) using a binomial generalized linear regression model with a logit link function—'glmer' function in 'lme4' package in R statistical software v. 3.2.1 (R Core Team 2015). We included all the fruit traits as fixed effects and the species as random effects and included species identity to pair ripe from unripe attributes by species. We examined all covariates for outliers and collinearity prior to analysis and \log_{10} transformed fruit mass and VOC to remove conspicuous outliers in the untransformed data. Fruit mass was correlated with both VOC and puncture resistance ($R^2 = 0.38$ and 0.56 , respectively), but variance inflation factors were <3 indicating no significant collinearity among the covariates and the global model (i.e., binomial regression model including all covariates) was not over-dispersed (dispersion parameter = 1.27). Accordingly, we included the fruit mass of ripe versus unripe fruits in the model. For the final analysis we standardized all covariates because the 'glmer' function works best on rescaled variables.

We evaluated the importance of the covariates as signals of fruit ripeness in two ways. First we examined the significance of the regression coefficient values in the global model. We accepted P values <0.1 as indicative of variables that were biologically important. Second, we conducted a model averaging procedure based on AIC_c , a second order form of AIC appropriate for both large and small sample sizes (Burnham and Anderson 2002), to obtain the weight of evidence or relative importance (w_+) values for each of the explanatory covariates. The w_+ is calculated as the sum of the Akaike weights (w_i) for all of the models in which the effect was present (Burnham and Anderson 2002). w_+ represents the probability that a given variable would be included in the best model. Variables with a reasonable level of support as predictors have a $w_+ \geq 0.73$; this is equivalent to an AIC_c difference of two units between the models containing the variable under examination and those not containing it (Richards 2005).

(2) *Random forest regression tree*: To test if fruit traits of species that were ripe differed from those that were unripe, and to visualise thresholds (Murray et al. 2011), we also constructed a random forest regression tree (Breiman 2001) for ripe and unripe fruits together. It is not possible to apply the random effect of species and thus the trait thresholds for ripe versus unripe fruits are not based on data paired by species. This method builds a classification

tree by repeatedly splitting the data based on whether they fall above or below a threshold value of each explanatory variable in the model (Bielby et al. 2010; Biau 2012). Because this method identifies interactions in which the same variable repeatedly enters a model at different levels, it can find threshold values (including if there are both upper and lower thresholds) (Davidson et al. 2009; Bielby et al. 2010).

The relative strength of association of covariates with the response variable can be difficult to interpret, because small changes in values of the covariates can alter their order in the tree (Bielby et al. 2010). To minimize this possibility and improve classification accuracy, our random forest approach combined a large number of regression trees and evaluated the results by a cross-validation process (Murray et al. 2011). Error is reported as an out-of-sample prediction error rate, in which prediction accuracy is determined on a subset of the data different from that used to generate the prediction. We used the package ‘randomForest’ in R (Breiman 2001; Liaw and Wiener 2002). To visualize the results of this analysis, we present a conditional inference tree based on the variables identified as the most strongly associated with the response variable by the random forest analysis. The tree was constructed using the function ‘ctree’ in the R package ‘Party’ (Hothorn et al. 2006).

Results

Phylogenetic Analysis

When mapped onto a model phylogeny (Fig. S1), none of the measured fruit traits show any evidence of being phylogenetically informative (Table 1). In general, CI and RI were much lower than would be sufficient for an informative character (Blomberg et al. 2003). Mean fruit weight and puncture resistance for both ripe and unripe fruits show the greatest homoplasy (CI = 0.553–0.699), but the other test statistics for these traits do not indicate a phylogenetic basis to their distributions. Additionally, we detected no significant phylogenetic structure for any trait using Blomberg’s K and Pagel’s λ (Table 1). We therefore conclude that these traits are not phylogenetically constrained. Accordingly, phylogeny was not included in the models.

Binomial Regression Analysis

The following predictions were supported based on the outcome of the binomial regression (Table 2; Fig. 1): (1) ripe fruits were significantly harder than unripe fruits; fruit puncture resistance was 0.27 ± 0.39 (mean \pm SD,

$n = 31$) and 0.53 ± 0.54 kg mm² for ripe and unripe fruit, respectively (Table 3), and had a relative importance ($w+$) of 0.93; and (2) paired by species, ripe fruit mass was significantly greater than unripe fruit mass (Table 3) and had high relative importance ($w+ = 0.86$). Although fruit odor and chromatic conspicuity had influence, and the predictions pertaining to these traits were supported, there were no significant differences between ripe and unripe fruits: (3) overall fruit odor (measured as VOC count) was higher in ripe (0.76 ± 0.64 ; mean \pm SD) than unripe fruits (0.59 ± 0.61), with $w+ = 0.49$; (4) chromatic conspicuity was greater in ripe (0.12 ± 0.08 ; mean \pm SD) versus unripe (0.09 ± 0.07 ; mean \pm SD) fruits, with $w+ = 0.48$; and lastly, (5) counter to our prediction that luminance conspicuity would be greater in ripe versus unripe fruits, we found the reverse to be true. Mean ripe fruit luminance conspicuity was 0.138 ± 0.092 , and mean unripe fruit luminance conspicuity was 0.148 ± 0.116 , meaning luminance has very low relative importance in the models ($w+ = 0.27$) (Table 3). These results suggest that the strongest indicators of Madagascan fruit ripeness for dichromats are mass and hardness, followed by color and odor. In this sample, the variation in luminance between fruits and background leaves appears to offer little or no indication of fruit ripeness.

Random Forest Regression Tree

The out of sample error rate in the regression tree analysis was large (38.7 %) and thus the trait thresholds were not significant and provide a guide rather than definitive thresholds. Nevertheless, the broad findings of the regression tree analysis were in agreement with the binomial regression approach. Fruit puncture resistance was the most important predictor of fruit ripeness (the first split, Fig. 2). Almost all fruits with a puncture resistance of ≤ 0.125 kg mm² were ripe and those that weren’t were chromatically conspicuous (≤ 0.123). Although there was a great deal of uncertainty associated with traits that describe unripe fruits, the most important of these traits was fruit mass; lighter fruit were generally unripe and among the heavier fruits, ripe fruits were best determined by their chromatic conspicuity (Fig. 2).

Discussion

We assessed potential indicators of fruit ripeness to seed dispersers in a Madagascan forest. Overall, only two of our five predictions were supported: ripe fruits had lower puncture resistance, and greater mass than unripe fruits. Contrary to our predictions, fruit odor, luminance, and chromatic conspicuity were not significantly greater in ripe

Table 1 Results from analyses of fruit traits using a model species-level phylogeny

	CI	RI	K	<i>p</i>	λ	<i>p</i>
Ripe						
Blue-yellow chromatic contrast	0.093	0.191	0.26	0.61	0.0001	1
Luminance contrast	0.16	0.138	0.219	0.78	0.0001	1
Mean fruit weight (g)	0.697	0.189	0.25	0.36	0.0001	1
Mean puncture resistance (kg/mm ²)	0.553	0.183	0.209	0.88	0.0001	1
VOC	0.239	0.042	0.327	0.66	0.0001	1
Unripe						
Blue-yellow chromatic contrast	0.112	0.187	0.28	0.47	0	1
Luminance contrast	0.204	0.273	0.253	0.61	0.0001	1
Mean fruit weight (g)	0.699	0.152	0.251	0.34	0.0001	1
Mean puncture resistance (kg/mm ²)	0.614	0.221	0.208	0.88	0.0001	1
VOC	0.29	0.178	0.356	0.56	0.098	0.74

P values for Blomberg's *K* and Pagel's λ are from likelihood-ratio tests

Table 2 Regression coefficients for fruit traits

Fruit trait	Estimate	Std. Error	z value	Pr(> z)	w+
Intercept (model)	−0.03428	0.29442	−0.116	0.9073	
Chroma	0.58519	0.33666	1.738	0.08217	0.477
Luminance	−0.35268	0.34001	−1.037	0.29961	0.274
Puncture resistance	−1.1891	0.51971	−2.288	0.02214	0.933
Mass	1.52049	0.57647	2.638	0.00835	0.857
VOC	0.8209	0.48306	1.699	0.08925	0.487

Fruit mass and VOC were log₁₀ transformed prior to analysis. All variables were standardized. Puncture resistance declines and fruit mass increases significantly with fruit ripeness. There is a strong trend of increasing fruit chroma and odor with fruit ripeness. These results are supported by the weight of evidence values (*w*+)

w+ is the probability of a given variable occurring in the best model and, therefore, reflects the weight of evidence of a relationship between the response and the given variable. Variables with a reasonable level of support as predictors (*w*+ ≥ 0.73) are shown in bold

versus unripe fruits. Of all potential fruit signals and cues to dispersers, haptic indicators relating to hardness and mass were the only consistently reliable indicators of fruit ripeness.

Fruit hardness can be a mechanical defense against seed predation because it functions to protect undeveloped, non-viable seeds from consumption (Kinzey and Norconk 1990; Lambert et al. 2004). The tissues surrounding the seeds of fruits act to restrict access by unwanted predators or ineffective seed dispersers, and likewise act as a nutritive reward for most intended animal dispersers (Lucas and Corlett 1998). Although it is likely that fruit hardness evolved to serve a protective function, softening of the exocarp is a consistent characteristic of fruit maturation (Dominy 2004) that may also function as a reliable indicator of ripeness to seed dispersing frugivores. Relatively few studies have quantified how dispersers rely on haptic indicators during foraging, however, anecdotal evidence reveals that primates palpate fruits during selection and

have been observed manually dehiscing fruits a few days before natural dehiscence occurs (Dominy et al. 2001). One study found that hardness of both fruit and pericarp might play a significant role in food choice among sympatric vertebrates (Kinzey and Norconk 1990). Unlike color and odor, the literature on haptic signals is limited and tends to focus on oral assessments of texture (van Roosmalen 1985; Corlett 2011). Digital assessment of fruit quality by mammalian seed dispersers has been rarely described. Interestingly, this is the indicator that we found to be salient across all fruits sampled: it is a consistent and reliable indicator of fruit ripeness. Further research on the role of fruit handling by frugivores in the field, coupled with experimental evidence of fruit choice as a function of haptic indicators would help to resolve the role of this variable.

Our finding that ripe fruits are heavier, and by implication, larger than unripe fruits is not surprising, as ripe fruits contain a higher water content and greater tissue

Fig. 1 Regression coefficient values based on standardized variables, and their 95 % CL, illustrating the importance of the fruit traits as indicators of fruit ripeness. The mass and puncture resistance of ripe fruits (relative to the unripe fruit of the same species) are significant at the <0.05 level, while chromaticity and odor (VOC) are important at the <0.1 level

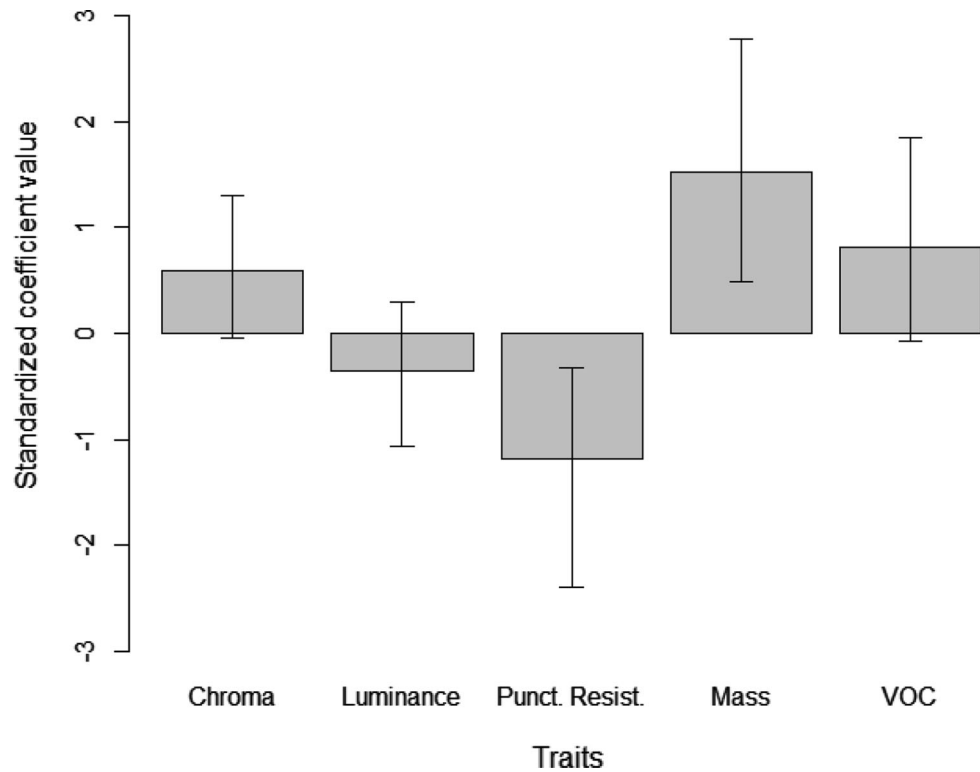


Table 3 Comparison of paired *t* test analyses of fruit traits

Trait	Unripe	Ripe	t_{30}	<i>P</i>
Chroma	0.091 ± 0.066	0.116 ± 0.079	-1.59	0.123
Luminance	0.148 ± 0.116	0.138 ± 0.092	0.037	0.714
Puncture resistance	0.535 ± 0.541	0.274 ± 0.396	3.637	0.001
Log ₁₀ Mass	0.155 ± 0.969	0.223 ± 0.923	-2.214	0.035
Log ₁₀ VOC	0.595 ± 0.612	0.758 ± 0.639	-2.823	0.008

P values in bold indicate significant differences between ripe and unripe fruits

volume than unripe fruits. Fruit size has also been previously identified as an important variable in primate foraging behavior (Florchinger et al. 2010). One study found that fruit morphologies of 69 Malagasy plant taxa dispersed by lemurs correlated significantly with fruit size (Dew and Wright 1998). Fruit size can be both a haptic and a visual signal/cue, and emphasizes the importance of vision and touch in assessing fruit quality. Along with fruit hardness, we found that fruit size was a consistent predictor of fruit ripeness in a Malagasy forest. Furthermore, in a sister study, fruit size was found to be the only significant predictor of fruit foraging efficiency for *E. fulvus* (Valenta et al. 2015a).

Our finding that overall VOC emissions of unripe fruits were not significantly lower than those of ripe fruits is surprising, especially in light of previous studies on lemur-dispersed fruits. One experimental study of the role of odor in Malagasy frugivore foraging found that *Microcebus*

murinus could identify ripe fruit based on odor in the absence of any other sensory stimuli (Siemers et al. 2007). Additionally, Valenta et al. (2013) found that overall odors of lemur-dispersed fruits are higher than that of non-lemur-dispersed fruits. Odors comprise more than overall smell signal, and the odor plume may not be as important in predicting foraging behavior as specific odorant compounds (Nevo et al. 2015). It is possible that frugivores are sensitive to one or more specific compounds within overall odor emissions. Thus, we cannot rule out the potential importance of odor as a reliable indicator of fruit ripeness. Further investigations of specific odor compounds in Malagasy fruits and their roles in attracting potential seed dispersers should be conducted.

That chromatic conspicuity between fruits and background leaves was not significantly higher in ripe than in unripe fruits is also surprising, because it suggests that chromatic conspicuity is not a reliable indicator of fruit

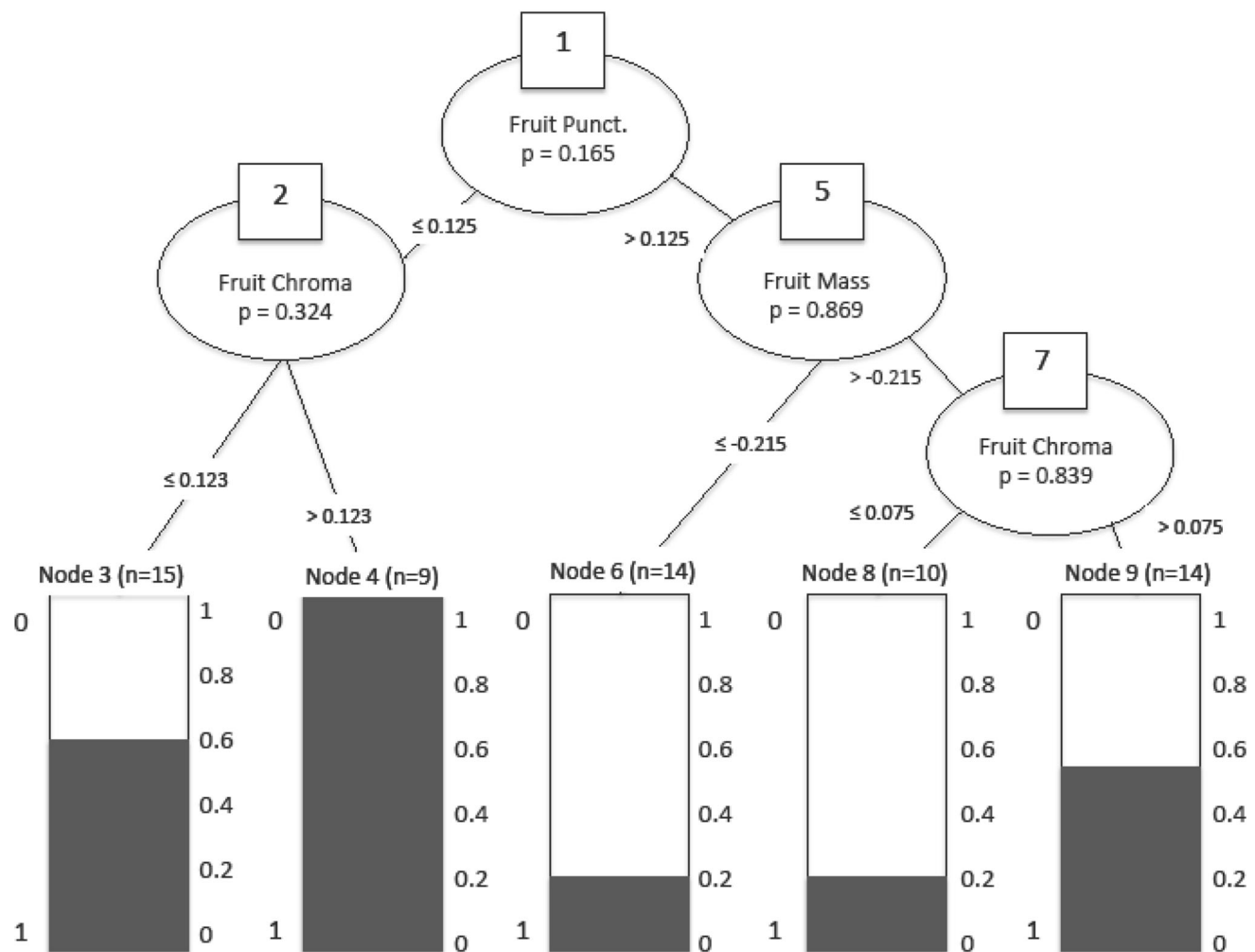


Fig. 2 Conditional inference tree based on the fruit traits most strongly associated with fruit ripeness from a random forest model. Shading represents the proportion of species that are ripe, and n is the number of species in each of the final groups. Numbers in boxes represent the node number at which each split occurred. Overall out-of-sample prediction error rate (overall misclassification rate) was

38.7%. Ripe fruits are first distinguished by their hardness (node 1) and any unripe fruits not captured by this split are identified by their chromatic conspicuity (node 2). Unripe fruits (within species) are identified by their lower mass (node 5) and then by their lower chromatic conspicuity (node 7)

ripeness in this Malagasy forest, at least to dichromatic mammals. Chromaticity has long been hypothesized to assist visual searching for fruits in tropical forests (e.g. Regan et al. 2001), and several studies have associated the evolution of trichromatic vision in tropical frugivorous vertebrates with increased foraging efficiency on colorful fruits (Regan et al. 1998; Cazetta et al. 2009). The degree of fruit ripeness in many plant species is also sometimes discernable by dichromatic primates (Sumner and Mollon 2000). While there is less evidence that color is a critical signal/cue to dichromats than trichromats, one study found that fruits of species dispersed by dichromatic mouse lemurs (*Microcebus* spp.) have higher chromatic contrast with background leaves than fruits of non-dispersed species (Valenta et al. 2013). In addition, some studies of

trichromatic vertebrates have corroborated our results. For example, in bird vision, fruits were not more contrasting to their own backgrounds than to those of other plant species, implying that fruit colors were not adapted for maximum conspicuousness for avian seed dispersers (Schaefer et al. 2007). Additionally, Sumner and Mollon (2000) found that the spectral positions of the primate long- and middle-wavelength cone pigments are not optimized for maximizing the chromatic distance between samples of different plant species. Thus, the evidence for the reliability of fruit chromaticity as an indicator of ripeness is mixed, and we have found that chromatic conspicuity is not a reliable forest-wide signal/cue to dichromatic mammals in our study system. Further investigations into chromaticity in other forest ecosystems would provide information about

the consistency and reliability of fruit color as an indicator of fruit ripeness.

Interestingly, unripe fruits are more visually conspicuous to dichromats in the luminance channel than are ripe fruits, though the difference is not significant. A study of neotropical primates found that foraging rates among species were positively correlated with fruit luminance conspicuity (Hiramatsu et al. 2008). Here, we compare ripe to unripe fruits of the same species, so the results of our study are not directly comparable. However, we were surprised to find that luminance contrast was actually higher for unripe fruits than ripe fruits. Because luminance contrast was high for both ripe and unripe fruits, it is possible that luminance contrast may allow animals to identify potential targets (i.e. any type of fruit), but not to differentiate ripe from unripe fruits.

Overall, only haptic indicators—hardness and mass—consistently indicate fruit ripeness to dichromatic lemurs during foraging. The quantification of multiple fruit traits offers an interesting and novel means of assessing the level of mutual dependence of fruits and frugivores. The significance of size and fruit hardness suggests that touch is an underappreciated sense, poorly represented in the literature, and of potential importance to frugivore foraging behavior. Although the question of plant-animal co-evolution and the existence of fruit signals is controversial (Lomascolo et al. 2010), the recent capacity to quantify fruit variables, such as chromaticity and odor, and to model these in biologically meaningful ways represents a novel approach to address the degree of overlap between animal sensory phenotypes and plant traits. Future research that includes animal-dispersed fruits in other forests where disperser guilds maintain different sensory phenotypes (e.g., continental Africa, the Neotropics, Southeast Asia), could further elucidate the broader parameters of animal-plant relations.

Compliance with Ethical Standards

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

References

- Allen, G. (1879). *The colour-sense: Its origin and development*. London: Trubner.
- Backlund, M., Oxelman, B., & Bremer, B. (2000). Phylogenetic relationships within the Gentianales based on *ndhF* and *rbcL* sequences, with particular reference to the Loganiaceae. *American Journal of Botany*, *87*, 1029–1043.
- Barton, R. A., Purvis, A., & Harvey, P. H. (1995). Evolutionary radiation of visual and olfactory brain systems in primates, bats and insectivores. *Philosophical Transactions of the Royal Society of London B*, *348*, 381–392.
- Bianconi, G. V., Suckow, U., Cruz-Neto, A. P., & Mikich, S. B. (2012). Use of fruit essential oils to assist forest regeneration by bats. *Restoration Ecology*, *20*, 211–217.
- Biau, G. (2012). Analysis of a random forests model. *Journal of Machine Learning Research*, *13*, 1063–1095.
- Bielby, J., Cardillo, M., Cooper, N., & Purvis, A. (2010). Modelling extinction risk in multispecies data sets: Phylogenetically independent contrasts versus decision trees. *Biodiversity and Conservation*, *19*, 113–127.
- Blomberg, S. P., Garland, T., Jr., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*, 717–745.
- Bollen, A. (2007). Fruit characteristics: fruit selection, animal seed dispersal and conservation matters in the Sainte Luce forests. In J. U. Ganzhorn, S. M. Goodman, & M. Vincelette (Eds.), *Biodiversity, ecology, and conservation of littoral ecosystems in the region of Tolagnaro (Fort Dauphin), southeastern Madagascar* (pp. 127–145). Washington DC: Smithsonian Institution.
- Brady, C. J. (1987). Fruit ripening. *Annual Review of Plant Physiology*, *38*, 155–178.
- Breiman, L. (2001). Random forests. *Machine Learning*, *45*, 5–32.
- Bremer, B., & Eriksson, T. (2009). Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and tribes. *International Journal of Plant Science*, *170*, 766–793.
- Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*. New York: Springer.
- Carvalho, L. S., Davies, W. L., Robinson, P. R., & Hunt, D. M. (2012). Spectral tuning and evolution of primate short-wave-length-sensitive visual pigments. *Proceedings of the Royal Society of London, B*, *279*, 387–393.
- Cazetta, E., Schaefer, H. M., & Galetti, M. (2009). Why are fruits colorful? The relative importance of achromatic and chromatic contrasts for detection by birds. *Evolutionary Ecology*, *23*(2), 233–244.
- Chapman, C. A., & Chapman, L. (1996). Frugivory and the fate of dispersed and non-dispersed seeds of six African tree species. *Journal of Tropical Ecology*, *12*, 491–504.
- Chase, M. W., & Reveal, J. L. (2009). A phylogenetic classification of the land plants to accompany APG III. *Botanical Journal of the Linnean Society*, *161*, 122–127.
- Corlett, R. T. (2011). How to be a frugivore (in a changing world). *Acta Oecologia*, *37*, 1–8.
- Dambroski, H. R., Linn, C. E., Jr, Berlocher, S. H., Forbes, A. A., Roelofs, W. L., & Feder, J. L. (2005). The genetic basis for fruit odor discrimination in *Rhagoletis* flies and its significance for sympatric host speciation. *Evolution*, *59*, 1953–1964.
- Davidson, A. D., Hamilton, M. J., Boyer, A. G., Brown, J. H., & Ceballos, G. (2009). Multiple ecological pathways to extinction in mammals. *Proceedings of the National Academy of Sciences*, *106*, 10702–10705.
- Dew, J. L., & Wright, P. (1998). Frugivory and seed dispersal by four species of primates in Madagascar's eastern rainforest. *Biotropica*, *30*, 425–437.
- Dominy, N. J. (2004). Fruits, fingers, and fermentation: The sensory cues available to foraging primates. *Integrative and Comparative Biology*, *44*, 295–303.
- Dominy, N. J., Lucas, P. W., Osorio, D., & Yamashita, N. (2001). The sensory ecology of primate food perception. *Evolutionary Anthropology*, *10*, 171–186.
- Farris, J. S. (1989). The retention index and the rescaled consistency index. *Cladistics*, *5*, 417–419.
- Fischer, K., & Chapman, C. (1993). Frugivores and fruit syndromes: Differences in patterns at the genus and species level. *Oikos*, *66*, 472–482.
- Florchinger, M., Braun, J., Bohning-Gaese, K., & Schaefer, H. M. (2010). Fruit size, crop mass, and plant height explain differential fruit choice of primates and birds. *Oecologia*, *164*, 151–161.

- Gadek, P. H., Fernando, E. S., Quinn, C. J., Hoot, S. B., Terrazas, T., Sheahan, M. C., & Chase, M. W. (1996). Sapindales: Molecular delimitation and infraordinal groups. *American Journal of Botany*, *83*, 802–811.
- Ganzhorn, J. U., Fietz, J., Rakotovo, E., Schwab, D., & Zinner, D. (1999). Lemurs and the regeneration of dry deciduous forest in Madagascar. *Conservation Biology*, *13*, 794–804.
- Gautier-Hion, A., Duplantier, J. M., Quris, R., Feer, F., Sourd, C., Decoux, J. P., et al. (1985). Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. *Oecologia*, *65*, 324–337.
- Gilad, Y., Wiebe, V., Przeworski, M., Lancet, D., & Paabo, S. (2004). Loss of olfactory receptor genes coincides with the acquisition of full trichromatic vision in primates. *PLoS Biology*, *2*, 120–125.
- Goff, S. A., & Klee, H. J. (2006). Plant volatile compounds: Sensory cues for health and nutritional value? *Science*, *311*(5762), 815–819.
- Goloboff, P. A., Farris, J. S., & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics*, *24*, 774–786.
- Herrera, C. M. (1985). Determinants of plant-animal coevolution: The case of mutualistic dispersal of seeds by vertebrates. *Oikos*, *44*, 132–141.
- Hiramatsu, C., Melin, A. D., Aureli, F., Schaffner, C. M., Vorobyev, M., Matsumoto, Y., & Kawamura, S. (2008). Importance of achromatic contrast in short-range fruit foraging of primates. *PLoS One*, *3*, e3356.
- Hirsch, B. T. (2010). Tradeoff between travel speed and olfactory food detection in ring-tailed coatis (*Nasua nasua*). *Ethology*, *116*, 671–679.
- Hothorn, T., Hornik, K., & Zeileis, A. (2006). Unbiased recursive partitioning: A conditional inference framework. *Journal of Computational and Graphical Statistics*, *15*, 651–674.
- Howe, H. F., & Smallwood, J. (1982). Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, *13*, 201–228.
- Jacobs, G. H., & Deegan, J. F. (1993). Photopigments underlying color vision in ringtail lemurs (*Lemur catta*) and brown lemurs (*Eulemur fulvus*). *American Journal of Primatology*, *30*, 243–256.
- Jacobson, M. (1978). *Handbook of sensory physiology: Development of sensory systems*. New York: Springer.
- Janson, C. H. (1983). Adaptation of fruit morphology to dispersal agents in a neotropical forest. *Science*, *219*, 187–189.
- Jordano, P. (1995). Angiosperm fleshy fruits and seed dispersers: A comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist*, *145*, 163–191.
- Kinzey, W. G., & Norconk, M. A. (1990). Hardness as a basis of fruit choice in two sympatric primates. *American Journal of Physical Anthropology*, *81*, 5–15.
- Knight, R. S., & Siegfried, W. R. (1983). Inter-relationships between type, size, and colour of fruits and dispersal in southern African trees. *Oecologia*, *56*, 405–412.
- Korine, C., & Kalko, E. K. V. (2005). Fruit detection and discrimination by small fruit-eating bats (Phyllostomidae): Echolocation call design and olfaction. *Behavioral Ecology and Sociobiology*, *59*, 12–23.
- Lahann, P. (2007). Biology of *Cheirogaleus major* in a littoral rain forest in southeast Madagascar. *International Journal of Primatology*, *28*, 895–905.
- Lambert, J. E., Chapman, C. A., Wrangham, R. W., & Conklin-Brittain, N. L. (2004). The hardness of cercopithecine foods: Implications for the critical function of enamel thickness in exploiting fallback foods. *American Journal of Physical Anthropology*, *125*, 363–368.
- Langrand, O. (1990). *Guide to the birds of Madagascar*. New Haven: Yale University Press.
- Laska, M., Liesen, A., & Teubner, P. (1999). Enantioselectivity of odor perception in squirrel monkeys and humans. *American Journal of Physiology*, *277*, 1098–1103.
- Liaw, A., & Wiener, M. (2002). Classification and regression by Random Forest. *R News*, *2*, 18–22.
- Linn, C. E., Jr, Dambroski, H. R., Feder, J. L., Berlocher, S. H., Nojima, S., & Roelofs, W. L. (2004). Postzygotic isolating factor in sympatric speciation in *Rhagoletis* flies: reduced response of hybrids to parental host-fruit odors. *Proceedings of the National Academy of Sciences*, *101*, 17753–17758.
- Lomascolo, S., Levey, D., Kimball, R., Bolker, B., & Alborn, H. (2010). Dispersers shape fruit diversity in *Ficus* (Moraceae). *Proceedings of the National Academy of Sciences*, *107*, 14668–14672.
- Lucas, P. W., & Corlett, R. T. (1998). Seed dispersal by long-tailed macaques. *American Journal of Primatology*, *45*, 29–44.
- Melin, A. D., Fedigan, L. M., Hiramatsu, C., & Kawamura, S. (2008). Polymorphic color vision in white-faced capuchins (*Cebus capucinus*): Is there foraging niche divergence among phenotypes? *Behavioral Ecology and Sociobiology*, *62*, 659–670.
- Melin, A. D., Moritz, G. L., Fosbury, R. A., Kawamura, S., & Dominy, N. J. (2012). Why aye-ayes see blue. *American Journal of Primatology*, *74*, 185–192.
- Mittermeier, C. G., Louis, E. E., Richardson, M., Schwitzer, C., Langrand, O., Rylands, A. B., et al. (2010). *Lemurs of Madagascar*. Bogota: Conservation International.
- Murray, K. A., Rosauer, D., McCallum, H., & Skerratt, L. F. (2011). Integrating species traits with extrinsic threats: closing the gap between predicting and preventing species declines. *Proceedings of the Royal Society B: Biological Sciences*, *278*, 1515–1523.
- Nazar, N., Goyder, D. J., Clarkson, J. J., Mahmood, T., & Chase, M. W. (2012). The taxonomy and systematics of Apocynaceae: Where we stand in 2012. *Botanical Journal of the Linnean Society*, *171*, 482–490.
- Nevo, O., Garri, R. O., Salazar, L. T. H., Schulz, S., Heymann, E. W., Ayasse, M., et al. (2015). Chemical recognition of fruit ripeness in spider monkeys (*Ateles geoffroyi*). *Scientific Reports*, *5*.
- Olson, V. A., & Owens, I. P. F. (1998). Costly sexual signals: Are carotenoids rare, risky or required? *Trends in Ecology and Evolution*, *13*, 510–514.
- Osorio, D., Smith, A. C., Vorobyev, M., & Buchanan-Smith, H. M. (2004). Detection of fruit and the selection of primate visual pigments for color vision. *American Naturalist*, *164*, 696–708.
- Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*, 2042–2051.
- Otte, D. (1974). Effects and functions in the evolution of signaling systems. *Annual Review of Ecology and Systematics*, *5*, 385–417.
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, *401*, 877–884.
- Paliyath, G., Murr, D. P., Handa, A. K., & Lurie, S. (2008). *Postharvest biology and technology of fruits, vegetables and flowers*. Singapore: Wiley.
- Peichl, L., Rakotondraparany, F., & Kappeler, P. M. (2001). Photoreceptor types and distributions in nocturnal and diurnal Malagasy primates. *Investigations in Ophthalmology and Visual Science*, *42*, 270.
- R Core Team. (2015). *R: A language and environment for statistical computing, version ver. 3.2.1*. Vienna: R Foundation for Statistical Computing.
- Regan, B. C., Julliot, C., Simmen, B., Vienot, F., & Charles-Dominique, P. (2001). Fruits, foliage, and the evolution of primate colour vision. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences*, *356*, 229–283.

- Regan, B. C., Julliot, C., Simmen, B., Vienot, F., Charles-Dominique, P., & Mollon, J. D. (1998). Frugivory and colour vision in *Alouatta seniculus*, a trichromatic platyrrhine monkey. *Vision Research*, 38(21), 3321–3327.
- Revell, L. J. (2012). Phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223.
- Richards, S. A. (2005). Testing ecological theory using the information-theoretic approach: Examples and cautionary results. *Ecology*, 86, 2805–2814.
- Rouquier, S., & Giorgi, D. (2007). Olfactory receptor gene repertoires in mammals. *Mutation Research*, 616, 95–102.
- Sanchez, F., Korine, C., Steeghs, M., Laarhoven, L., Cristescu, S. M., Harren, F. J. M., et al. (2006). Ethanol and methanol as possible odor cues for egyptian fruit bats (*Rousettus aegypticus*). *Journal of Chemical Ecology*, 32, 1289–1300.
- Schaefer, H., & Braun, J. (2009). Reliable cues and signals of fruit quality are contingent on the habitat in black elder (*Sambucus nigra*). *Ecology*, 90, 1564–1573.
- Schaefer, H., Schaefer, V., & Vorobyev, M. (2007). Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *The American Naturalist*, 169, S159–S169.
- Schatz, G. E. (2001). *Generic tree flora of madagascar*. St. Louis: Royal Botanic Gardens.
- Schupp, E. W. (1993). Quantity, quality and the effectiveness of seed dispersal by animals. In T. H. Fleming & A. Estrada (Eds.), *Frugivory and seed dispersal: Ecological and evolutionary aspects* (pp. 15–29). Netherlands: Springer.
- Siemers, B. M., Goerlitz, H. R., Robsomanitrdrasana, E., Piep, M., Ramanamanjato, J. B., Rakotodravony, D., et al. (2007). Sensory basis of food detection in wild *Microcebus murinus*. *International Journal of Primatology*, 28, 291–304.
- Sumner, P., & Mollon, J. D. (2000). Chromaticity as a signal of ripeness in fruits taken by primates. *The Journal of Experimental Biology*, 203, 1987–2000.
- Tan, Y., & Li, W. H. (1999). Trichromatic vision in prosimians. *Nature*, 402, 36.
- Tokuoka, T., & Tobe, H. (2006). Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceae sens. str. *Journal of Plant Research*, 119, 599–616.
- Valenta, K., Brown, K. A., Melin, A. D., Monckton, S. K., Styler, S. A., Jackson, D. A., & Chapman, C. A. (2015a). It's not easy being blue: are there olfactory and visual trade-offs in plant signalling? *PLoS One*, 10, e0131725.
- Valenta, K., Brown, K. A., Rafaliarison, R. R., Styler, S. A., Jackson, D., Lehman, S. M., et al. (2015b). Sensory integration during foraging: the importance of fruit hardness, colour, and odour to brown lemurs. *Behavioral Ecology and Sociobiology*, 69, 1855–1865.
- Valenta, K., Burke, R. J., Styler, S. A., Jackson, D. A., Melin, A. D., & Lehman, S. M. (2013). Colour and odour drive fruit selection and seed dispersal by mouse lemurs. *Scientific Reports*, 3, 1–5.
- Valenta, K., Edwards, M., Rafaliarison, R. R., Johnson, S. E., Holmes, S., Brown, K., et al. (2015c). Visual ecology of true lemurs suggests a cathemeral origin for the primate cone opsin polymorphism. *Functional Ecology*.
- Valido, A., Schaefer, H. M., & Jordano, P. (2011). Colour, design and reward: Phenotypic integration of fleshy fruit displays. *Journal of Evolutionary Biology*, 24, 751–760.
- van Roosmalen, M. G. M. (1985). Habitat preferences, diet, feeding strategy and social organization of the black spider monkey (*Ateles paniscus paniscus* Linnaeus 1758) in Surinam. *Acta Amazon*, 15, 12–38.
- Wheelwright, N. T., & Janson, C. H. (1985). Colors of fruit displays of bird-dispersed plants in two tropical forests. *American Naturalist*, 126, 777–799.