

## Research



**Cite this article:** Nevo O, Valenta K, Razafimandimby D, Melin AD, Ayasse M, Chapman CA. 2018 Frugivores and the evolution of fruit colour. *Biol. Lett.* **14**: 20180377.  
<http://dx.doi.org/10.1098/rsbl.2018.0377>

Received: 22 May 2018

Accepted: 5 September 2018

### Subject Areas:

ecology, evolution, plant science

### Keywords:

animal–plant interactions, coevolution, colour vision, mutualism, seed dispersal, sensory ecology

### Author for correspondence:

Omer Nevo

e-mail: omer.nevo@evolutionary-ecology.de

<sup>†</sup>Equal contribution.

Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4227983>.

## Evolutionary biology

# Frugivores and the evolution of fruit colour

Omer Nevo<sup>1,†</sup>, Kim Valenta<sup>2,†</sup>, Diary Razafimandimby<sup>3</sup>, Amanda D. Melin<sup>4,5,6</sup>, Manfred Ayasse<sup>1</sup> and Colin A. Chapman<sup>7,8,9</sup>

<sup>1</sup>Institute of Evolutionary Ecology and Conservation Genomics, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

<sup>2</sup>Department of Evolutionary Anthropology, Duke University, 130 Science Dr., Durham, NC 27708, USA

<sup>3</sup>Faculty of Sciences, Zoology and Animal Biodiversity, University of Antananarivo, Antananarivo, Madagascar

<sup>4</sup>Department of Anthropology and Archaeology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

<sup>5</sup>Department of Medical Genetics, and <sup>6</sup>Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Alberta, Canada T2N 1N4

<sup>7</sup>McGill School of the Environment, Department of Anthropology, McGill University, 855 Sherbrooke St. W, Montreal, Canada H3A 2T7

<sup>8</sup>School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, KwaZulu-Natal 3209, South Africa

<sup>9</sup>Key Laboratory of Resource Biology and Biotechnology in Western China of Ministry of Education, and College of Life Sciences, Northwest University, Xi'an 710069, China

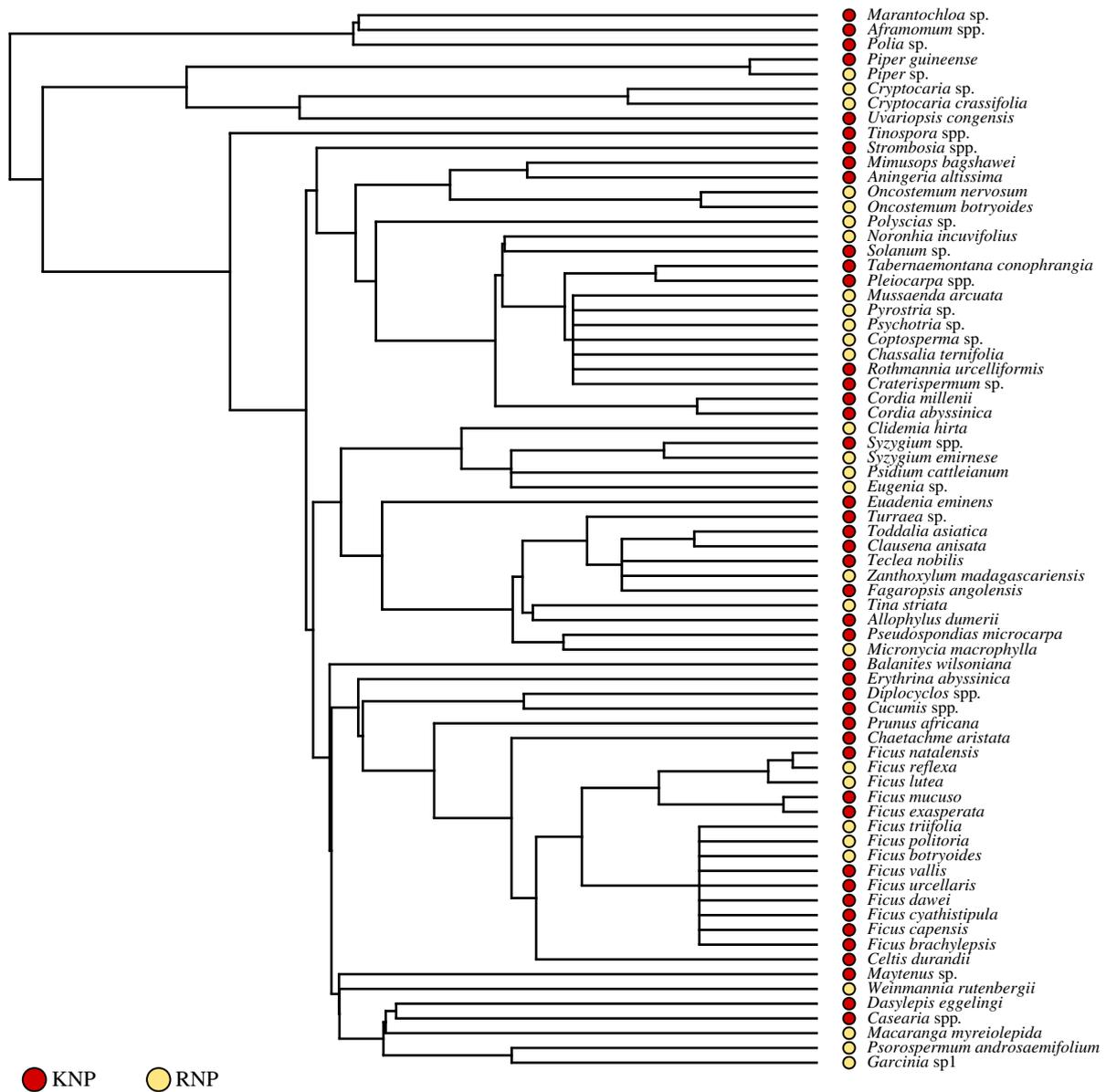
ON, 0000-0003-3549-4509; CAC, 0000-0002-8827-8140

The ecological function of fruit colour has been the focus of many studies. The most commonly tested hypothesis is that fruit colour has evolved to facilitate detection by seed-dispersing animals. We tested whether distributions of fruit colours are consistent with the hypothesis that colour is an evolved signal to seed dispersers using a comparative community approach. We compared the contrast between ripe fruits and leaf backgrounds at two sites, one in Madagascar where seed dispersers are primarily night-active, red–green colour-blind lemurs, and the other in Uganda, where most vertebrate seed dispersers are day-active primates and birds with greater capacity for colour vision. We show that fruits in Uganda have higher contrast against leaf background in the red–green and luminance channels whereas fruits in Madagascar contrast more in the yellow–blue channel. These results indicate that fruit colour has evolved to contrast against background leaves in response to the visual capabilities of local seed disperser communities.

## 1. Introduction

The degree to which fruit traits result from selection by animal mutualists is a long-debated question [1–4]. Among the myriad fruit traits, colour has received the most attention, perhaps because of its diversity and conspicuousness to human observers. Fruit colour has been hypothesized to increase fruit conspicuousness to seed dispersers by resulting in a visual contrast against backgrounds, primarily mature leaves [5–7]. The debate regarding the adaptive significance of fruit colour is far from being settled [3]. Some studies have found that colour is not likely to be under selection by seed dispersing animals [1,8,9]. Others found that when other relevant factors are controlled for, there is evidence that frugivores have driven the evolution of fruit colour, and that colour may be subject to weaker constraints than other fruit traits [2,3,10,11].

Discrepancies between studies are the result of several factors. Some rely on subjective colour categories recorded by human observers [1,9] despite the fact that the human colour-vision phenotype is rare and shared with only Old-World monkeys and apes [12]. Additionally, some studies consider fruit colour in isolation [9], even though the locus of selection is not colour *per se*,



**Figure 1.** Phylogeny of study species. Red—Kibale National Park, Uganda (KNP). Yellow—Ranomafana National Park, Madagascar (RNP). Phylogeny is from Zanne *et al.* [13]. Representatives of the same families and genera are present in both sites.

but rather the visual contrast between fruit and its background [7]. Finally, many studies compare fruits dispersed exclusively by a single group of animals [2], which precludes identifying variation in fruit colour resulting from selection pressures exerted by multiple different frugivores [3]. While some community-level studies have been conducted, they compared communities in which the differences in frugivore visual capacities are not fully known, or not sufficiently diverse to generate differences in fruit coloration [8,9].

Here, we employ a comparative approach to test the hypothesis that fruit colour has evolved in response to seed disperser visual phenotypes. We compare fleshy fruits from two plant communities: Kibale National Park (KNP), Uganda, and Ranomafana National Park (RNP), Madagascar. Both sites are well studied, representative, and relatively undisturbed montane rainforests in similar elevations, which host plant taxa from the same genera and families (figure 1) but very different frugivore communities (electronic supplementary material, table S1). KNP supports a frugivore community of primarily diurnal, trichromatic primates and tetrachromatic birds that can readily discriminate between reds and greens [14]. In contrast, RNP is dominated by

mainly nocturnal or cathemeral lemurs [15,16], many of which cannot distinguish reds and greens, and all of which can distinguish contrasts on the yellow–blue channel. As a result, most KNP species are dispersed by uniformly trichromatic primates and tetrachromatic birds, while most RNP species are either exclusively or partially dispersed by dichromatic or polymorphic lemurs (electronic supplementary material, table S1). Thus, we predicted that if fruit colour is selected to maximize conspicuousness to frugivores, ripe fruit–leaf contrasts would be higher in KNP, except in the yellow–blue colour channel that is accessible to most RNP frugivores. We calculated chromatic and achromatic contrast between fruits and background leaves in 72 species from the two sites, and used phylogenetically controlled models to compare visual conspicuousness of fruits against background leaves in three channels: red–green (visible to trichromatic and tetrachromatic observers), yellow–blue, and luminance.

## 2. Material and methods

Ripe fruits and mature leaves of 44 plant species (figure 1; electronic supplementary material, table S1) from KNP were

collected between May 2015 and Dec 2016. We focused on fleshy fruits that are dispersed by local birds and mammals, primarily primates, though two species from KNP are also dispersed by elephants. Samples of 28 plant species from RNP (figure 1; electronic supplementary material, table S1) were collected between Oct 2016 and Sep 2017. Fruits were considered ripe when they changed their colour and were known to be consumed by local frugivores. One to twenty fruits (mean 5.2 per individual) and leaves from 1 to 10 individuals (mean 1.92 per species) per species were collected and immediately brought to the field laboratory for processing. KNP fruit and leaf colour was quantified using a Jaz portable spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics Inc.) emitting a D-65 light source, with optical probes fixed at a 45 degree angle. RNP samples were analysed using the same reflectance standard, light source and sampling parameters, with a USB2000+UV-VIS miniature fibre optics spectrometer (Ocean Optics). Measurement parameters were identical in the two study sites. For each species, we calculated the mean reflectance in each 0.42 nm bin across all samples and individuals.

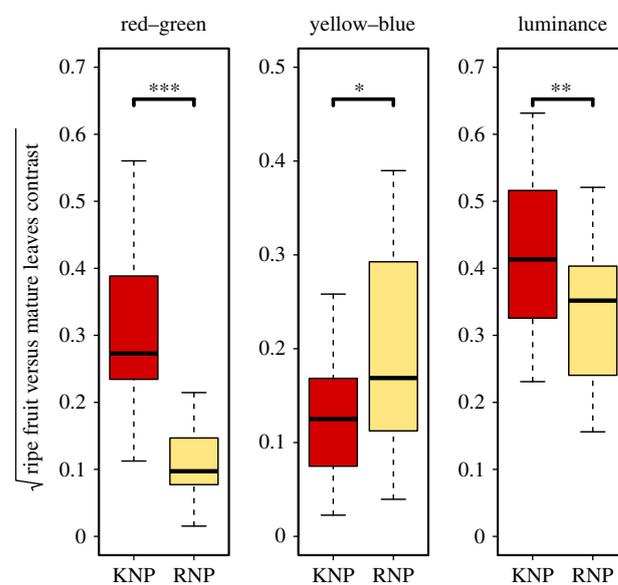
For each plant species, we calculated chromatic and achromatic contrast between fruits and background leaves under a diurnal, forest shade illuminant in three visual channels: red–green, yellow–blue, and luminance, following Hiramatsu *et al.* [17]. To allow direct comparison, we simulated a representative trichromatic phenotype based on S cones, M cones and L cones, with peak spectral sensitivities ( $\lambda_{\max}$ ) of 425, 535 and 562 nm, respectively. These peak sensitivities roughly correspond to the typical catarrhine phenotype, as well as many strepsirrhines [18]. All diurnal birds would also be able to perceive the three colour channels reported here [19]. Dichromatic phenotypes, which are common in Madagascar [15], would be able to perceive the yellow–blue and luminance contrasts, but not red–green contrasts. We compared visual contrasts between fruits and leaves in all three channels using phylogenetic generalized least square analyses (PGLS) and a phylogeny by Zanne *et al.* [13]. We used three models, in which contrast (red–green, yellow–blue, or luminance) was the response variable and study site (KNP, RNP) was a single predictor variable. All continuous variables were square-root transformed to comply with the statistical test's assumptions. All analyses were conducted in R v. 3.4.3 [20] using packages APE [21], Phytools [22] and nlme [23].

### 3. Results

PGLS models revealed that fruits in KNP have a significantly higher contrast against mature leaves in the red–green (PGLS; d.f. = 72, 70;  $t = -9.79$ ,  $p < 0.001$ ) and luminance (PGLS; d.f. = 72, 70;  $t = -2.83$ ,  $p < 0.01$ ) channels (figure 2) than fruits in RNP. In contrast, in the yellow–blue channel, fruits in RNP contrast against background leaves significantly more than in KNP (PGLS; d.f. = 72, 70;  $t = 2.16$ ,  $p < 0.05$ ) (figure 2).

### 4. Discussion

Based on the trichromatic visual models we used, fruits in KNP are significantly more visually conspicuous to local frugivores (primates and birds) than fruits in RNP in the red–green and luminance channels. This is especially noticeable in the former, where the median contrast in RNP scored lower than the lowest-contrasting fruit in KNP (figure 2). In contrast, RNP fruits are more visually conspicuous in the yellow–blue channel, although the difference between the sites is smaller than the differences in the other channels. These patterns of fruit coloration correspond to differences



**Figure 2.** Contrasts between ripe fruits and mature leaves. KNP—Kibale National Park (Uganda). RNP—Ranomafana National Park (Madagascar). Contrast on each channel was calculated based on the visual model of representative trichromatic local primates. Contrasts in all tests were square-root transformed to meet the assumptions of the statistical tests used.  $N = 72$  species in all analyses. Asterisks denote significance at  $\alpha < 0.05$  (\*),  $\alpha < 0.01$  (\*\*) and  $\alpha < 0.001$  (\*\*\*) in a PGLS model using a phylogeny by Zanne *et al.* [13].

in the typical colour-vision phenotype of their respective frugivore communities. In Madagascar, most lemurs are dichromats (red–green colour blind) and visual conspicuousness can only be achieved through contrast in the yellow–blue and luminance channels. In contrast, trichromatic primates and tetrachromatic birds in KNP can readily distinguish reds and greens, and it has recently been shown that red–green discrimination leads to higher fruit intake rates in trichromatic primates [24]. The phylogenetic similarities in plant communities, i.e. that closely related species are found at the different geographical sites (figure 1), imply that between-site differences cannot be attributed to common ancestry. Thus, these results strongly support the hypothesis that fruit colour is shaped by the visual phenotypes of seed disperser communities [2,10,25].

While the differences in the red–green and yellow–blue channels were expected, the higher achromatic contrast (luminance) in KNP requires further explanation, because luminance can be useful for fruit detection by dichromats and trichromats [26]. A reasonable explanation is that while RNP frugivores may use fruit colour as a foraging and food selection cue, they also tend to rely more strongly on olfactory cues [27]. Thus, Malagasy fruits may be under weaker selection pressures to offer visually-conspicuous fruits. Moreover, the frugivore community of KNP is more diverse and includes many different primates and birds, whose colour discrimination capacities and foraging strategies may vary. For example, fruit-foraging birds rely on chromatic and achromatic (luminance) cues in different situations [28]. Thus, the higher contrast in luminance, along with high red–green contrast and non-negligible contrast in the yellow–blue channel, may reflect high overall colour conspicuousness as an adaptation to dispersal by a diverse community of visually oriented frugivores. If so, these results highlight the importance of considering the role of multiple

frugivores in shaping fruit characteristics. While past studies have compared primate and bird-dispersed fruits [2], our results suggest that the joint effect of frugivores with red–green colour discrimination capacity (trichromatic primates and primarily tetrachromatic birds) can yield patterns that become clear once compared with communities in which these colour-vision phenotypes are less common. Acknowledging that these conclusions are based on a comparison of two sites, we encourage future studies to test the hypothesis using other sites in Madagascar, mainland Africa, and other localities.

In conclusion, using a comparative community approach, our results support the hypothesis that fruit colour is under selection exerted by colour-vision phenotypes of seed dispersers. These results are in accordance with several previous studies [2,10], and disagree with the conclusions of a more recent work [9]. An overarching pattern—that more visually conspicuous fruits are found in areas with visually oriented frugivores—emerges when quantifying and modelling colour,

focusing on the locus of selection, and comparing communities that are sufficiently diverse in their frugivore communities to generate community-wide differences in fruit phenotypes.

**Data accessibility.** Electronic supplementary material, table S1 contains all raw data used in the manuscript.

**Authors' contributions.** O.N. acquired funding, collected data, designed the study, analysed data and wrote the manuscript. K.V. collected data, designed the study and wrote the manuscript. D.R. collected data. A.D.M. analysed data and wrote the manuscript. M.A. acquired funding, designed the study and helped writing the manuscript. C.A.C. acquired funding, designed the study and helped writing the manuscript. All authors have seen and approved the final version of this manuscript.

**Competing interests.** We declare no competing interests.

**Funding.** O.N. was funded by a German Science Foundation grant (NE 2156/1-1) while working on this manuscript. C.A.C. was supported by NSERC Canada and the CRC program.

**Acknowledgements.** We thank Patricia Wright, the Wenner Gren Foundation and Centre ValBio for providing the instrument used for data collection in RNP.

## References

- Fischer KE, Chapman CA. 1993 Frugivores and fruit syndromes: differences in patterns at the genus and species level. *Oikos* **66**, 472–482. (doi:10.2307/3544942)
- Lomáscolo SB, Schaefer HM. 2010 Signal convergence in fruits: a result of selection by frugivores? *J. Evol. Biol.* **23**, 614–624. (doi:10.1111/j.1420-9101.2010.01931.x)
- Valenta K, Nevo O, Chapman CA. 2018 Primate fruit color: useful concept or alluring myth? *Int. J. Primatol.* **39**, 321–337. (doi:10.1007/s10764-018-0025-y)
- Jordano P. 1995 Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant–animal interactions. *Am. Nat.* **145**, 163–191. (doi:10.1086/285735)
- Cazetta E, Schaefer HM, Galetti M. 2007 Why are fruits colorful? The relative importance of achromatic and chromatic contrasts for detection by birds. *Evol. Ecol.* **23**, 233–244. (doi:10.1007/s10682-007-9217-1)
- Schaefer HM, Ruxton GD. 2011 *Animal–plant communication*. Oxford, UK: Oxford University Press.
- Schaefer HM, Schmidt V. 2004 Detectability and content as opposing signal characteristics in fruits. *Proc. R. Soc. Lond. B* **271**, S370–S373. (doi:10.1098/rsbl.2004.0191)
- Burns KC, Cazetta E, Galetti M, Valido A, Schaefer HM. 2009 Geographic patterns in fruit colour diversity: do leaves constrain the colour of fleshy fruits? *Oecologia* **159**, 337–343. (doi:10.1007/s00442-008-1227-3)
- Brodie JF. 2017 Evolutionary cascades induced by large frugivores. *Proc. Natl Acad. Sci. USA* **114**, 11998–12002. (doi:10.1073/pnas.1710172114)
- Schaefer HM, Valido A, Jordano P. 2014 Birds see the true colours of fruits to live off the fat of the land. *Proc. R. Soc. B* **281**, 20132516. (doi:10.1098/rspb.2013.2516)
- Renoult JP, Valido A, Jordano P, Schaefer HM. 2014 Adaptation of flower and fruit colours to multiple, distinct mutualists. *New Phytol.* **201**, 678–686. (doi:10.1111/nph.12539)
- Jacobs GH. 2009 Evolution of colour vision in mammals. *Phil. Trans. R. Soc. B* **364**, 2957–2967. (doi:10.1098/rstb.2009.0039)
- Zanne AE *et al.* 2014 Three keys to the radiation of angiosperms into freezing environments. *Nature* **506**, 89–92. (doi:10.1038/nature12872)
- Struhsaker TT. 1997 *Ecology of an African rain forest: logging in Kibale and the conflict between conservation and exploitation*. Gainesville, FL: University Press of Florida.
- Valenta K *et al.* 2016 Visual ecology of true lemurs suggests a cathemeral origin for the primate cone opsin polymorphism. *Funct. Ecol.* **30**, 932–942. (doi:10.1111/1365-2435.12575)
- Wright PC, Razafindratsita VR, Pochron ST, Jernvall J. 2005 The key to Madagascar frugivores. In *Tropical fruits and frugivores: the search for strong interactors* (eds JL Dew, JP Boubli), pp. 121–138. Dordrecht, The Netherlands: Springer.
- Hiramatsu C, Melin AD, Aureli F, Schaffner CM, Vorobyev M, Matsumoto Y, Kawamura S. 2008 Importance of achromatic contrast in short-range fruit foraging of primates. *PLoS ONE* **3**, e3356. (doi:10.1371/journal.pone.0003356)
- Jacobs RL *et al.* 2017 Novel opsin gene variation in large-bodied, diurnal lemurs. *Biol. Lett.* **13**, 20170050. (doi:10.1098/rsbl.2017.0050)
- Bennett ATD, Théry M. 2007 Avian color vision and coloration: multidisciplinary evolutionary biology. *Am. Nat.* **169**, S1–S6. (doi:10.1086/510163)
- R Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <http://www.R-project.org/>.
- Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
- Revell LJ. 2012 Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223. (doi:10.1111/j.2041-210X.2011.00169.x)
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team. 2017 nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131. See <https://CRAN.R-project.org/package=nlme>.
- Melin AD, Chiou KL, Walco ER, Bergstrom ML, Kawamura S. 2017 Trichromacy increases fruit intake rates of wild capuchins (*Cebus capucinus imitator*). *Proc. Natl Acad. Sci. USA* **114**, 10 402–10 407. (doi:10.1073/pnas.1705957114)
- Schaefer HM, Schaefer V, Levey DJ. 2004 How plant–animal interactions signal new insights in communication. *Trends Ecol. Evol.* **19**, 577–584. (doi:10.1016/j.tree.2004.08.003)
- Osorio D, Vorobyev M. 2005 Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. B* **272**, 1745–1752. (doi:10.1098/rspb.2005.3156)
- Nevo O, Valenta K. 2018 The ecology and evolution of fruit odor: implications for primate seed dispersal. *Int. J. Primatol.* **39**, 338–355. (doi:10.1007/s10764-018-0021-2)
- Schaefer HM, Levey DJ, Schaefer V, Avery ML. 2006 The role of chromatic and achromatic signals for fruit detection by birds. *Behav. Ecol.* **17**, 784–789. (doi:10.1093/beheco/arl011)