New Phytologist

Research

# How colorful are fruits? Limited color diversity in fleshy fruits on local and global scales

**Kalliope E. Stournaras[1,2], Eugenia Lo[2], Katrin Böhning-Gaese[3,4], Eliana Cazetta[5], D. Matthias Dehling[3], Matthias Schleuning[3], Mary Caswell Stoddard[6], Michael J. Donoghue[2], Richard O. Prum[2] and H. Martin Schaefer[1]**

[1]Department of Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg, Hauptstrasse 1, 79104, Freiburg, Germany; [2]Department of Ecology and Evolutionary Biology, Yale University, PO Box 208105, New Haven, CT 06520, USA; [3]Biodiversity and Climate Research Centre (BiK-F) & Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, 60325, Frankfurt/Main, Germany; [4]Department of Biological Sciences, Goethe Universität, Max-von-Laue-Straße 9, 60438, Frankfurt/Main, Germany; [5]Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Ilhéus-Itabuna km 16, Ilhéus, Bahia, CEP 45662-900, Brazil; [6]Museum of Comparative Zoology and Department of Organismic & Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

Author for correspondence:
*Kalliope E. Stournaras*
*Tel: +49 761 2032559*
*Email: k.stournaras@gmx.de*

Received: *31 October 2012*
Accepted: *18 December 2012*

*New Phytologist* (2013) **198:** 617–629
**doi**: 10.1111/nph.12157

**Key words:** angiosperm phylogeny, color gamut, color space, constraints, flower color, fruit color diversity, null model, seed disperser.

## Summary

- The colors of fleshy fruits are considered to be a signal to seed-dispersing animals, but their diversity remains poorly understood. Using an avian color space to derive a sensory morphospace for fruit color, we tested four hypotheses of fruit color diversity: fruit colors occupy a limited area of the color space; they are less diverse than flower colors; fruit colors within localities are similar to each other; and fruit color diversity reflects phylogeny.
- The global fruit color diversity of 948 primarily bird-dispersed plant species and the color diversity of localities were compared with null models of random, unconstrained evolution of fruit color. Fruit color diversity was further compared with the diversity of 1300 flower colors. Tests of phylogenetic effects on fruit color were used to assess the degree of correspondence with phylogeny.
- Global and local fruit color diversity was limited compared with null models and fruits have achieved only half the color diversity of flowers. Interestingly, we found little indication of phylogenetic conservatism.
- Constraints resulting from the chemical properties of pigments probably limit global fruit and flower color diversity. Different types of selection on fruits and flowers may further explain the smaller color diversity of fruits.

## Introduction

The color of fleshy fruits is considered to be a signal to seed-dispersing animals that promotes the detection of fruits and the dispersal of seeds. Although the past decades have seen various studies on this plant–animal communication system and, particularly, on fruit color (Snow, 1971; Willson & Whelan, 1990; Schaefer & Schmidt, 2004; Schmidt *et al.*, 2004; Burns *et al.*, 2009; Schaefer & Ruxton, 2011; Valido *et al.*, 2011), the diversity and evolution of fruit color are still poorly understood. Although some studies have reported fleshy fruits dispersed mainly by birds as being red or black, others have marveled at the bewildering color diversity of these fruits (Wheelwright & Janson, 1985; Wheelwright, 1988; Willson *et al.*, 1989). Our lack of understanding of fruit color diversity also reflects the lack of hypotheses that specifically aim to explain fruit color diversity. The only hypothesis tested so far, the disperser syndrome hypothesis, is controversial (Janson, 1983; Fischer & Chapman, 1993; Lomáscolo *et al.*, 2010). It states that differential selection from distinct dispersers (e.g. birds, mammals) partly explains some of the differences in the colors of fruits dispersed by these dispersers. This hypothesis, however, cannot explain the color diversity of fruits dispersed by the same type of disperser. We therefore lack a framework to predict the color diversity of fruits dispersed by one type of disperser.

Theoretical morphospaces provide an elegant approach to study both the spectrum of forms found in nature and the selective pressures and constraints that have shaped it during evolution (McGhee, 1999, 2007). Morphospaces typically are geometric spaces which represent all theoretically possible morphological shapes and into which existing forms can be mapped. Both filled and empty areas of a morphospace are valuable for the study of biological forms. The filled areas represent the range of realized forms in nature, whereas the empty areas allow the exploration of whether: constraints (e.g. biochemical, developmental, etc.) bias against the production of some forms; selection is acting against some forms; some forms simply have not (yet) evolved; or some forms are impossible to produce in nature (e.g. a leaf that is 100 m long and 1 mm wide; McGhee, 1999). Although constraints can differ between organisms and evolution can find ways to overcome them, impossible forms are a global phenomenon. Theoretical morphospaces can be built not only for geometric forms, but for all quantitative traits. For example, instead of placing morphological variables on the axes of the space, other variables, such as visual receptor responses of animals, can be used to build receptor spaces for color (Endler & Mielke, 2005; Stoddard & Prum, 2008, 2011). Because the sensory abilities of perceivers mediate the evolution of signals in communication systems (Endler *et al.*, 2005), such sensory color spaces are highly suitable for the biological study of color signals. As yet, however, they have not been used to study the diversity of color signals in plant–animal communication.

In the present work, we investigate fruit color diversity using an avian sensory color space. Because birds act as important seed dispersers for many plant species (Jordano, 2000), their visual system constitutes an appropriate sensory space for the study of fleshy fruit color (hereafter fruit color). Moreover, among the visually oriented seed dispersers, birds possess the visual system with the highest color discrimination abilities. Birds have excellent tetrachromatic color vision, with four different cone types ranging in their absorption between 300 and 700 nm, bestowing them with a high color definition (Bowmaker *et al.*, 1997). A three-dimensional sensory color space can be built on the basis of the relative excitations of the four cone types. All colors visible to birds can be mapped into the color space depending on how strongly they excite each of the four cone types (Goldsmith, 1990). Because distance in the color space is probably a good predictor of perceived difference in color, quantitative metrics for color diversity, such as the volume of filled areas, have been developed (Endler & Mielke, 2005; Stoddard & Prum, 2008). Importantly, the avian sensory color space is, per definition, a finite space with a finite volume, so that both fruit color diversity and the unoccupied regions of color space can be quantified.

Generally, existing forms do not fill out morphospaces, but leave extensive areas empty (McGhee, 2007; Stoddard & Prum, 2011). This can be a result of any combination of the above-mentioned reasons (constraints, selection, not yet evolved forms, impossible forms). For example, a large number of bird plumage colors, as well as plant colors (leaf, bark and flower colors), each fill out only *c.* 30% of the avian sensory color space, in part because natural colors cannot reach some color space areas that require very high excitation of just one or two cone types (impossible colors; Vorobyev, 2003; Stoddard & Prum, 2011). The same limitation is also likely to apply to fruit colors. In addition, although fruits originate developmentally from parts of flowers and may, in theory, be able to produce the same colors as flowers do, different types of selective pressure may be acting on fruit and flower colors. For example, although diversification in flower color and shape is probably strong to ensure repeated visits of pollinators (Gumbert *et al.*, 1999), fruits may profit more from having more similar colors (Schaefer *et al.*, 2004; Whitney, 2009). Such effects are expected to contribute to a lower color diversity in fruits relative to flowers (Whitney, 2009).

In this study, our aim is to provide a framework for the understanding of color diversity of bird-dispersed fruits by testing the following four hypotheses: (1) the fruit color gamut, that is, the global range of achieved fruit colors (*sensu* Sumner & Mollon, 2003; Stoddard & Prum, 2011), does not fill out the avian sensory color space, but occupies a restricted area (restricted gamut hypothesis); (2) the fruit color gamut does not achieve the color diversity of the flower color gamut (Whitney hypothesis); (3) fruit colors of localities are similar to each other, leading to a small local diversity (similarity hypothesis); (4) fruit colors can be accurately predicted from a knowledge of phylogenetic relationships (phylogenetic conservatism hypothesis). We test the restricted gamut hypothesis on a fruit gamut consisting of a large dataset of 948 mainly bird-dispersed plant species against a gamut null model of random, unrestricted color evolution in the color space. To address the Whitney hypothesis, we compare the fruit gamut diversity with the diversity of a flower color gamut consisting of 1300 flower colors. Further, we test the similarity hypothesis in 15 localities against a locality null model of random fruit color evolution in localities within the bounded fruit gamut color volume. Lastly, we investigate the phylogenetic conservatism hypothesis at two levels. We test for phylogenetic signal in fruit color using Blomberg's *K*. Further, we investigate how phylogenetic relationships affect fruit color diversity by testing whether fruit color diversity within angiosperm clades (in this case 'orders') differs from randomly assembled clades, and whether the overlap of the fruit color volume of major clades differs from the overlap of random clades.

## Materials and Methods

### Fruit reflectance data collection

Reflectance spectra of ripe, fleshy fruits from 948 plant species dispersed mostly by birds were included in the study. Fleshy fruits were collected at 15 localities, as well as a botanic garden. Variation in the spatial extent of sampled localities could affect the results on diversity; therefore, we included localities sampled at different geographic scales. Three of the localities corresponded to larger sampling regions (hereafter called regions), one locality included two communities and the remaining 11 each corresponded to a single community (hereafter called communities).

The three regions were southern Germany (hereafter 'Germany', $n = 59$), southern Spain (hereafter 'Spain', $n = 37$) and northern Florida (hereafter 'Florida', USA, $n = 27$). In Germany, fruits were collected in the Rhine valley and a region of the Black Forest around the city of Freiburg. In Spain, fruits were collected in the National Park of Doñana and in a valley in the Sierra de Cazorla. Fruits of the Florida region were collected in hardwood hammocks close to Gainesville.

The Cardoso locality (south-eastern Brazil, $n = 73$) included two communities (restinga and lowland tropical forest) in the

Atlantic rainforest area of Ilha do Cardoso (Sao Paulo State, Brazil). The remaining 11 localities each corresponded to a single community (Pantanal (western Brazil), $n = 38$; Kakamega Forest (western Kenya), $n = 40$; Amazonian Rainforest (Esmeralda, southern Venezuela), $n = 51$; and eight communities in Manu National Park and Biosphere Reserve in southeastern Peru: Pantiacolla 1, $n = 151$; Pantiacolla 2, $n = 55$; Tono, $n = 36$; San Pedro, $n = 157$; Trocha Union 1, $n = 50$; Trocha Union 2, $n = 46$; Wayqecha, $n = 103$; Tres Cruces, $n = 35$). Detailed information on sampling and localities can be found in Supporting Information Methods S1. Information on vegetation types of the Peruvian communities is summarized in Patterson *et al.* (1998). A summary of the sizes of the localities is provided in Table 1.

In each locality, all encountered ripe, fleshy fruits dispersed mostly by birds were collected regardless of fruit color. Birds are the most important seed dispersers for > 90% of the plant species in all localities, although mammals often also contribute to dispersal (Herrera, 1984; Schmidt *et al.*, 2004; Donatti *et al.*, 2007; Schaefer *et al.*, 2007; Flörchinger *et al.*, 2010; D.M. Dehling, pers. obs.). Between 10 and 20 ripe fruits per species were measured in the laboratory using an Ocean Optics USB2000 spectrometer (Ocean Optics, Dunedin, FL, USA) and a Top Sensor System Deuterium-Halogen DH-2000 (Top Sensor Systems, Eerbeek, the Netherlands) or a DT-MINI- GS-2 as standardized light source, or an AvaSpec 2048 spectrometer with a xenon light source (Avantes, Eerbeek, the Netherlands).

To further enrich the fruit color gamut, we also collected all ripe fleshy fruits dispersed by birds from the Botanic Garden of the University of Freiburg ($n = 65$). Twenty ripe fruits per species were measured in the laboratory with an Ocean Optics USB2000 spectrometer (Ocean Optics) and a Top Sensor System Deuterium-Halogen DH-2000 (Top Sensor Systems). This sample included species from all over the world. In addition, because of its rare color production mechanism, the published reflectance curve of *Elaeocarpus angustifolius* (Lee, 1991), which is native to Australasia, was included in the gamut. These samples were included in all analyses, except for the locality null model.

**Table 1** Summary of the sizes of the sampled localities in hectares

| Locality | Size (ha) |
|---|---|
| Germany | 90 000 |
| Spain | 112 500 |
| Florida | 20 000 |
| Cardoso | 2000 |
| Pantanal | 5000 |
| Kakamega Forest | 250 |
| Amazonian Forest | 5 |
| Pantiacolla 1 | 1 |
| Pantiacolla 2 | 1 |
| Tono | 1 |
| San Pedro | 2.4 |
| Trocha Union 1 | 1 |
| Trocha Union 2 | 1 |
| Wayquecha | 1.8 |
| Tres Cruces | 1 |

## Avian color space

Two types of visual system occur in birds, called UVS (ultraviolet sensitive) and VS (violet sensitive), depending on the sensitivity peak of the pigment type with the lowest absorption range, with UVS pigments absorbing maximally at lower wavelengths (Vorobyev & Osorio, 1998; Hart & Hunt, 2007). The derived UVS system offers a higher color resolution of fruits, flowers and natural objects compared with the ancestral VS system (Schaefer *et al.*, 2007; Osorio & Vorobyev, 2008; Stoddard & Prum, 2011). We therefore used the UVS system of the blue tit (*Cyanistes caeruleus*) for the avian color space, as it provides a biologically relevant sensory space with the highest ability to resolve color diversity.

We used an avian tetrahedral color space model as a sensory morphospace for fruit and flower color (Stoddard & Prum, 2008; TetraColorSpace, written in MATLAB 7 software, MathWorks, Natick, MA, USA). TetraColorSpace transforms reflectance spectra between 300 and 700 nm into relative (normalized to sum to one) cone stimulation values based on the sensitivity curves of the chosen avian visual system. Relative cone stimulations, denoted as $\{uv, s, m, l\}$, correspond to the relative excitations of the avian ultraviolet-sensitive (UVS), short-wavelength-sensitive (SWS), middle-wavelength-sensitive (MWS) and long-wavelength-sensitive (LWS) cones, respectively. Relative cone stimulation values are plotted in a tetrahedron in which each vertex corresponds to one of the four cone types (Stoddard & Prum, 2008). Thus, the reflectance spectrum of each species is represented as one single point (color point) in the avian color space. The closer the point lies to a particular vertex, the more strongly the respective cone type is stimulated. Reflectance spectra of fruits of the 948 plant species were imported into the avian color space and constituted our fruit color gamut.

We used two metrics that describe different aspects of color space occupancy: (1) the volume of the convex hull of a color sample (smallest possible polyhedron containing all points) which corresponds to the space occupied by the color points and is a measure of color diversity in the sensory space; (2) the variance in span, which is calculated as the variance of all pairwise Euclidian distances in a given color sample, and is a metric of how evenly spaced the color points are (Stoddard & Prum, 2008, 2011; Stoddard & Stevens, 2011).

## Effect of the UVS system on results

We chose the avian UVS visual system to conduct our analyses because it constitutes a biologically meaningful sensory space with the highest color resolution. To ascertain that our results were not artifacts of the chosen space, we repeated analyses in an avian VS color space (peafowl, *Pavo cristatus*). The results did not differ qualitatively (results not shown).

## Fruit gamut null model

We tested the restricted gamut hypothesis against a gamut null model of fruit color evolution which assumes that any color inside the color space can be produced and fruit colors evolve randomly in the color space. We chose this null model of random and unconstrained color evolution because it allows us to test precisely these processes. The second assumption is justifiable because fruit color probably is an evolutionary labile trait showing low phylogenetic signal (Lomáscolo & Schaefer, 2010). The results of the phylogenetic conservatism hypothesis strongly support this (see the Results section). We created samples of 948 (sample size of fruit gamut) random points drawn without replacement (i.e. same color point not allowed more than once in each sample; 1000 iterations) from a random (uniform) distribution inside the avian color space and calculated the volume of each sample. We compared the color volume of the fruit gamut with the 95% confidence interval of the random volume distribution (interval between 2.5 and 97.5 quantiles of random distribution obtained by random sampling; referred to as 95% CI).

Because any point in the avian color space corresponds to a set of four values $\{uv, s, m, l\}$ with $uv + s + m + l = 1$, the creation of a random point in the color space requires four random numbers that sum to one. Throughout our study, we used a 'broken stick' method to create random color points in the color space: three random numbers drawn from a uniform distribution between zero and one were generated and ranked so that $a < b < c$. The numbers $a$, $b$ and $c$ were then treated as the breaking points of the stick, resulting in $uv = a$, $s = b - a$, $m = c - b$ and $l = 1 - c$.

## Comparison of fruit and flower gamut

We tested the Whitney hypothesis by comparing the diversities (volume of convex hull) of the fruit and flower gamut. The flower color gamut consisted of 1300 flower reflectance spectra between 300 and 700 nm from 876 plant species downloaded from the Floral Reflectance Database (FReD; Arnold *et al*., 2010) and analyzed in the avian sensory space. The species originated from all over the world and are pollinated by a range of organisms. All flower spectra available at the database at the time were downloaded; spectra were excluded if > 25 reflectance entries were equal to zero. Spectra belonging to the same species that looked identical to the eye were averaged.

Flowers often have multiple colors, and so the flower gamut contains more color points than species. We investigated whether the fruit and flower gamuts achieve the same color diversity. To achieve this, we calculated the volume of each gamut and the overlap between the volumes of the fruit and flower gamut. Volume overlap was calculated using a method derived from Stoddard & Stevens (2011). The shared volume of the fruit and flower gamut was expressed as a percentage number relative to each gamut. Further, to account for the fact that the two gamuts might have different phylogenetic diversities, which might affect the comparison of their color diversities, we repeated the color overlap calculation only for species of genera found in both datasets ($n = 29$ genera, with $n = 143$ fruit species and $n = 89$ flower species).

In addition, to identify fruit colors that are not represented in the flower gamut and vice versa, we calculated a density factor for each fruit and flower color point. For every color point of each gamut, we calculated how many points of the other gamut fell inside a sphere centered at the focal point and with a volume of 1% of the color space. The number of points inside that sphere gave the density at each focal point.

Because of differences in sample sizes (1300 flower vs 948 fruit colors), direct comparisons between the diversity of the two gamuts might be difficult. To assess the degree to which the two samples are comparable, we applied a procedure derived from the ecological rarefaction method (Sanders, 1968). By randomly drawing one after another point from the gamut and calculating the volume of the increasing sample of points (no replacement; 200 iterations), we obtained a color volume curve (comparable with a rarefaction curve) for each gamut as a function of the number of color points in the sample. Comparison of the slope of the two curves allowed an estimate of how complete the sampling of each gamut is.

## Locality null model

We tested the similarity hypothesis against a locality null model which assumes that fruits can realize any color within the fruit gamut volume, and fruit colors of a locality evolve randomly inside the fruit gamut volume leading to random fruit color diversity. As noted above, the assumption of random color evolution is justifiable because fruit color shows very low phylogenetic signal (see the Results section). In order to achieve a random (uniform) distribution of colors within the fruit gamut volume, we needed to discard random color points that were outside the convex hull of the gamut. However, if we use any $N$ points to define a gamut and create thousands of random points that fall exactly inside the convex hull of this gamut, samples of $N$ points from the randomly created points always have a significantly smaller average volume than the $N$ gamut. The same is true for $M$ points that are a subset of the $N$ points. The volume of the $M$ points is always larger than the average of 1000 randomly drawn samples of $M$ points from the created points inside $N$. This is because the outer points of the $N$ gamut define the surface of the convex hull, but the probability that random points will lie on the surface of the hull is extremely small. Thus, the creation of random points that fall exactly inside the convex hull systematically underestimates the color diversity of random samples.

To correct for this bias, we relaxed the condition that random points fall exactly inside the convex hull of the fruit color gamut. Each random point was allowed to increase the volume of the gamut $V_{\text{gamut}}$ by a factor $x$. The magnitude of this factor was determined iteratively, so that the average volume of 1000 random samples of 948 randomly created points was equal to $V_{\text{gamut}}$. This condition was satisfied for $x = 0.18\%$. Random points were generated and discarded if they increased $V_{\text{gamut}}$ by > 0.18%, until a total of 50 000 random color points (hereafter the random pool) had been created. The total volume of the random pool was 7.6% larger than $V_{\text{gamut}}$.

To test the similarity hypothesis, we compared the volume and variance in span of each of the 15 localities with that expected under the null model. Because each observed locality has the same number of species as the simulated ones, a difference in volume between observed and simulated localities implies a difference in the similarity between fruit colors. Likewise, the variance in span is a direct measure of the evenness of similarity of fruit colors in a sample.

To calculate the expected distributions, 5000 random localities with as many points as species in the respective fruit locality were repeatedly drawn without replacement from the random pool. For each random locality, the convex hull volume and variance in span were calculated, providing the expected null distributions for both metrics. For each expected distribution, we calculated 95% CIs. If the observed value was outside the 95% CI of the respective distribution, the observed value was considered to be significantly different from the null model (with $P < 0.05$).

One locality (Cardoso, Brazil, $n = 73$) yielded a result qualitatively different from the other localities when compared with the null model. This locality contained several species that at least visually seemed to occupy distinct areas in the gamut with no similar fruit colors close to them. To objectively quantify such potential outliers, we computed a density factor for each point in the gamut. Similar to the procedure described above, for each point in the fruit gamut, we calculated how many of the fruit gamut points were found inside the sphere with 1% volume. Plant species with density zero (no points except the focal point found inside the sphere) occupy the most sparsely populated areas of the gamut and are regarded as outliers in this study.

Five outlier species were found in the fruit gamut, four of which belonged to the Cardoso locality. To analyze the effect of these four points on locality color diversity, we also compared the Cardoso locality after excluding the outliers to the null model. In addition, we removed all five species with density zero from the gamut and repeated all locality null model calculations.

### Phylogenetic conservatism

We constructed a phylogeny of the species present in the fruit gamut using Phylomatic (Webb & Donoghue, 2005) and according to the latest Angiosperm Phylogeny Group (APG) classification (APG III, 2009). The output tree represents relationships among APG III family-level clades, as well as among some, but not all, of the genera within each of these clades (e.g. in Rosaceae and Rubiaceae, relationships among many genera were resolved). Relationships within genera were generally treated as polytomies. The samples included in this study belong to a total of 117 families, each containing 1–24 genera (mostly 10 or fewer). The number of species included per genus was relatively small (in the range 1–13). We assigned branch lengths using the BLADJ block in Phylocom (Webb *et al.*, 2008) to obtain an ultrametric tree used in testing for phylogenetic signal.

We tested the phylogenetic conservatism hypothesis in two ways. First, we tested for phylogenetic signal in fruit color, that is the degree to which phylogeny predicts similarities in the fruit colors of species. For this purpose, we used Blomberg's $K$ (Blomberg *et al.*, 2003), which measures phylogenetic signal when compared with a model of Brownian motion evolution on the phylogeny. Fruit color in the three-dimensional avian sensory space is accurately described by the three coordinates $x$, $y$ and $z$, which were entered as three traits into Blomberg's $K$ calculation. The analysis was carried out with the Picante package in R (Kembel *et al.*, 2010).

As many different underlying trait distribution patterns can be consistent with any particular value of Blomberg's $K$, we also tested whether order-level clades (APG III, 2009) differed in their fruit color diversity (volume) from random expectation. If so, this could affect the diversity observed in the communities in which they are present. Using our baseline phylogeny, we identified 18 orders with adequate species sampling (> 10 species; median, 27; minimum, 11; maximum, 133 species): Apiales, Aquifoliales, Arecales, Cornales, Dipsacales, Ericales, Fabales, Gentianales, Lamiales, Laurales, Magnoliales, Malpighiales, Myrtales, Ranunculales, Rosales, Santalales, Sapindales and Solanales. The fruit color diversity of orders expected by chance was calculated by reshuffling repeatedly the species at the tips of the phylogenetic tree (using MATLAB; tree topology remained constant; no replacement, 10 000 iterations) and calculating the color volume of resulting 'random orders'. The observed order color volumes were compared with the 95% CI of the random distributions.

Moreover, we tested whether the overlap in color volume of four much more inclusive clades (*sensu* Cantino *et al.*, 2007) identified in the phylogenetic tree (Asteridae, $n = 328$; Rosidae, $n = 423$; Monocotyledoneae, $n = 64$; Magnoliidae, $n = 79$) differed from a scenario of random phylogenetic relationships among species. We used the percentage overlap of the color volumes (convex hulls) of those four major clades to estimate how similar the fruit colors of species in these clades are.

Pairwise volume overlap was calculated for the four major clades and was expressed as a percentage relative to the volume of each clade, yielding a nonsymmetric pairwise volume overlap table. We calculated expected random volume overlap by reshuffling the species at the tips of the phylogenetic tree (using MATLAB; leaving tree topology constant; no replacement, 1000 iterations) and calculating the percentage pairwise volume overlap of the resulting random clades. The observed volume overlap was then compared with the 95% CI of the expected volume overlap distributions.

All analyses, except for Blomberg's $K$ calculations, were carried out in MATLAB 7 (MathWorks, Natick, MA, USA). Functions and scripts are available on request.

## Results

### Restricted color diversity of fruits

The fruit color gamut of 948 mostly bird-dispersed plant species occupied an absolute volume of 0.0363 inside the avian UVS sensory space (Fig. 1a). This volume is significantly smaller than expected by the gamut null model (95% CI of random distribution = 0.2011–0.2084) and corresponds to 16.8% of the avian color space, confirming the prediction of the restricted gamut hypothesis that fruit colors make use of only a small part of the color diversity visible to birds. Given that our fruit sample included chiefly bird-dispersed fruits, one should bear in mind that this might be a conservative estimate of the overall color

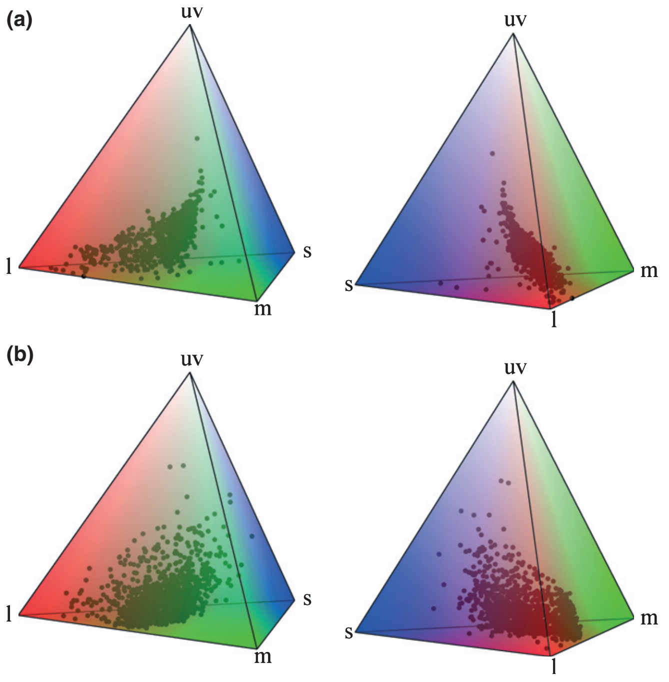

**Fig. 1** Fruit (a) and flower (b) color gamut in the avian sensory color space, viewed from two different angles. (a) Each point represents the fruit color of one plant species; (b) several flower color points can belong to the same species. Relative cone catches uv, s, m and l are maximized at the respective vertex of the tetrahedron. The color of the tetrahedron is a crude approximation for illustration purposes.

diversity of fleshy fruits. A short video of the fruit color gamut viewed in the rotating avian color space can be found in Supporting Information Video S1.

In the sampled fruit color gamut, 62.1% of all species show an excitation of the *l* cone that is stronger than each of the excitations of the *uv*, *s* and *m* cones, respectively, meaning that they appear 'red' or 'purplish-black' to birds. This figure supports the observation often stated in the literature that at least 60% of bird-dispersed fruits are either red or black in human color categories (Wheelwright & Janson, 1985).

## Fruit gamut is less diverse than flower gamut

The flower gamut of 1300 plant species occupied a volume of 0.0661, which corresponds to 30.5% of the avian sensory color space (Fig. 1b). Flowers, therefore, show almost twice the color diversity of fruits. Although 93.4% of the fruit gamut volume is also occupied by the flower gamut, only 53.7% of the flower gamut volume overlaps with the fruit gamut. Using the density factor on both the fruit and flower gamut, we identified flower color points that populate areas which the fruit gamut colors do not (Fig. 2). A total of 84 flower color points (6.5% of flower colors) are distinct from the fruit colors. By contrast, just two fruit color points are distinct from flower colors (0.2% of fruit colors). In other words, flowers produce almost all colors that fruits do. Many of the distinct flower points are characterized by a relatively high excitation of the *uv* cone, and some additionally by a relatively strong excitation of the *s* cone (saturated blue color to the human eye). Both distinct fruit colors have a high excitation of the *s* cone and very low excitation of the *uv* cone (Fig. 2).

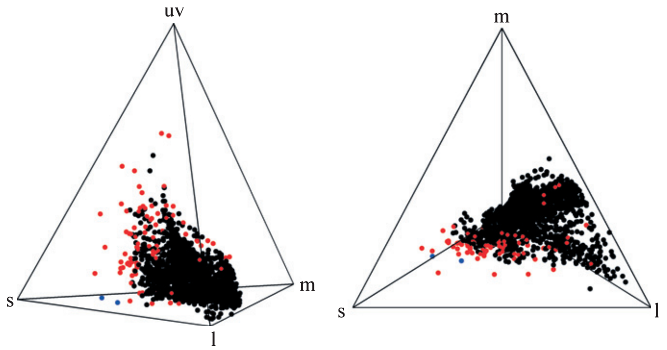

**Fig. 2** Density factor for the fruit and flower gamut, viewed from two different angles. Black points indicate fruit or flower colors with density one or greater. Points in red indicate unique flower colors with zero fruit color point density (unique flower colors). Blue points show fruit colors with zero flower color density (unique fruit colors). Relative cone catch values uv, s, m and l are maximized at the respective vertex of the tetrahedron.

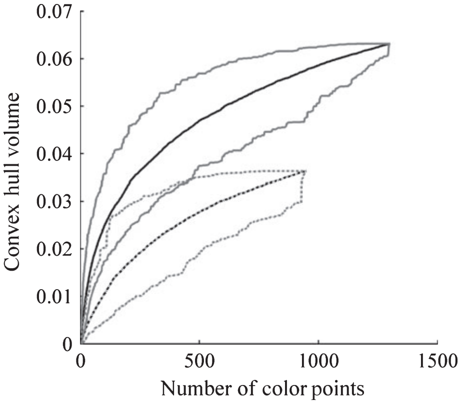

**Fig. 3** Color volume rarefaction curves of the flower gamut (black solid line) and the fruit gamut (black dotted line) with 95% CI (gray solid and dotted lines, respectively).

The volume rarefaction of the fruit and flower gamut supports the finding that flowers overall achieve a higher color diversity than fruits independent of sample sizes (Fig. 3). The 95% CI of the two curves do not overlap above *c.* 500 color points, indicating that flowers and fruits differ significantly in their achieved color diversities. Further, the volume of the common genera in fruits and flowers was 0.0157 for flowers and 0.013 for fruits. Dividing the normalized flower volume (flower volume of common genera/number of species in common genera) by the normalized fruit volume (fruit volume of common genera/number species in common genera) resulted in a ratio of 1.9. This ratio is surprisingly close to the global finding that flower colors are approximately twice as diverse as fruit colors, and thus strongly supports it.

## Color similarity in fruits of localities

All but one of the 15 localities sampled at different geographic scales exhibited significantly smaller than expected color volumes (Table 2). Only the volume of Cardoso was not significantly

**Table 2** Summary of comparisons between the locality null model and observed volume and variance in span of localities

| | Full fruit gamut | | | | | Truncated fruit gamut | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Color volume | | Variance in span | | | Color volume | | Variance in span | |
| Locality | *n* | Observed | 95% CI | Observed | 95% CI | *n* | Observed | 95% CI | Observed | 95% CI |
| Germany | 59 | *0.0058* | 0.0197–0.0276 | **0.0216** | 0.0098–0.0175 | 59 | *0.0058* | 0.0116–0.0160 | **0.0216** | 0.0081–0.0154 |
| Spain | 37 | *0.0033* | 0.0149–0.0242 | **0.0189** | 0.0091–0.0186 | 37 | *0.0033* | 0.0090–0.0138 | **0.0189** | 0.0074–0.0168 |
| Florida | 27 | *0.0061* | 0.0119–0.0218 | **0.0270** | 0.0082–0.0192 | 27 | *0.0061* | 0.0069–0.0125 | **0.0270** | 0.0066–0.0176 |
| Cardoso | 73 | 0.0285 | 0.0221–0.0286 | **0.0199** | 0.0104–0.0168 | 69 | *0.0073* | 0.0126–0.0164 | **0.0163** | 0.0085–0.0154 |
| Cardoso (no outliers) | 69 | *0.0073* | 0.0214–0.0283 | 0.0163 | 0.0102–0.0173 | | | | | |
| Pantanal | 38 | *0.0039* | 0.0154–0.0246 | 0.0094 | 0.0090–0.0189 | 38 | *0.0039* | 0.0091–0.0141 | 0.0094 | 0.0074–0.0164 |
| Kakamega Forest | 40 | *0.0058* | 0.0161–0.0246 | 0.0135 | 0.0091–0.0188 | 40 | *0.0058* | 0.0092–0.0142 | 0.0135 | 0.0073–0.0164 |
| Amazonian Rainforest | 51 | *0.0036* | 0.0187–0.0265 | 0.0122 | 0.0096–0.0180 | 51 | *0.0036* | 0.0108–0.0152 | 0.0122 | 0.0079–0.0156 |
| Pantiacolla 1 | 151 | *0.0026* | 0.0277–0.0321 | *0.0036* | 0.0113–0.0160 | 151 | *0.0026* | 0.0165–0.0191 | *0.0036* | 0.0095–0.0139 |
| Pantiacolla 2 | 55 | *0.0019* | 0.0198–0.0267 | *0.0060* | 0.0100–0.0176 | 55 | *0.0019* | 0.0114–0.0156 | *0.0060* | 0.0080–0.0155 |
| Tono | 36 | *0.0014* | 0.0147–0.0243 | *0.0049* | 0.0089–0.0183 | 36 | *0.0014* | 0.0091–0.0140 | *0.0049* | 0.0074–0.0168 |
| San Pedro | 157 | *0.0026* | 0.0280–0.0321 | *0.0030* | 0.0113–0.0161 | 157 | *0.0026* | 0.0167–0.0191 | *0.0030* | 0.0094–0.0140 |
| Trocha Union 1 | 50 | *0.0022* | 0.0183–0.0265 | *0.0042* | 0.0097–0.0180 | 50 | *0.0022* | 0.0108–0.0154 | *0.0042* | 0.0079–0.0161 |
| Trocha Union 2 | 46 | *0.0012* | 0.0173–0.0256 | *0.0028* | 0.0096–0.0179 | 46 | *0.0012* | 0.0101–0.0149 | *0.0028* | 0.0080–0.0162 |
| Wayquecha | 103 | *0.0021* | 0.0250–0.0304 | *0.0032* | 0.0110–0.0165 | 103 | *0.0021* | 0.0148–0.0179 | *0.0032* | 0.0089–0.0143 |
| Tres Cruces | 35 | *0.0006* | 0.0148–0.0238 | *0.0020* | 0.0089–0.0187 | 35 | *0.0006* | 0.0085–0.0137 | *0.0020* | 0.0071–0.0172 |

Numbers in bold indicate significantly larger, numbers in italics indicate significantly smaller and other numbers indicate no significant difference of the observed value relative to the 95% CI expected from the community null model. The truncated fruit gamut results after exclusion of the five outlier species. *n*, number of species in the respective community.

different from the expected null volume. Overall, fruit colors of localities are less diverse and more similar to each other than expected by chance, even when accounting for the observed limited global fruit color diversity. The result is qualitatively the same for 14 localities despite the fact that sampling ranges differed markedly among them. This result supports the similarity hypothesis. Results for variance in color span were less consistent across localities. The three regions as well as Cardoso showed a significantly higher than expected variance in span, whereas the observed variance of three communities did not differ from the null expectation. A higher than random variance in span means that fruit colors of these localities are less evenly distributed in the volume they occupy than expected by chance. This implies that, although some colors may be very similar, others are more distant. By contrast, all Peruvian communities had a significantly smaller variance in span than expected, showing that fruit colors are more evenly distributed in space than expected randomly.

## Effects of outliers on color diversity of localities

Using the density function, we identified five species with density zero, meaning that no other color from the gamut can be found inside the defined sphere (circumscribing 1% of the avian color space; Fig. 4, Table 3). The position of outlier species in the phylogeny is indicated in Supporting Information Fig. S1. Together, these five species accounted for 39.3% of the total fruit gamut volume (gamut volume before removal of five species, 0.0363; gamut volume after removal, 0.0221). Thus, the five species, hereafter referred to as outliers, occupy distinct areas in the color space and increase disproportionally the overall gamut diversity.

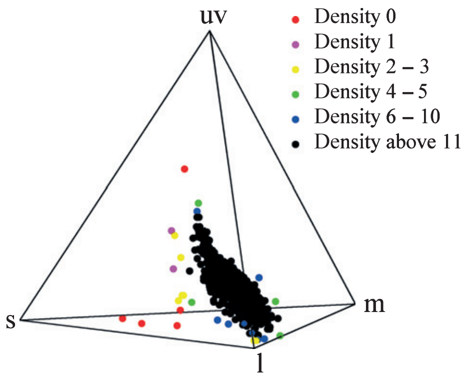

**Fig. 4** Density of color points in the fruit color gamut. Points with density zero (shown in red) are treated as outliers. Relative cone catch values uv, s, m and l are maximized at the respective vertex of the tetrahedron.

Four of the five identified outlier species were found only in Cardoso and accounted for 74.3% of the color diversity of this locality (volume before removal, 0.0285; volume after removal, 0.0073). By contrast, the removal of any four species from Cardoso at random resulted in an average volume reduction of 7% (minimum, 0%; maximum, 52%; 1000 iterations). After removing the four outlier species with disproportionate effects from Cardoso and recalculating the null model for that locality, we found that the color volume of the truncated locality became significantly smaller than expected under the null model (Table 2), and thereby comparable with the values of the other communities, whereas the variance in span was not found to differ from the expected variance.

**Table 3** Information on the five outlier species of the fruit color gamut

| Species | Family | Order* | Major clade | Community |
|---|---|---|---|---|
| *Elaeocarpus angustifolius* | Elaeocarpaceae | Oxalidales | Rosidae | † |
| *Heliconia velloziana* | Heliconiaceae | Zingiberales | Monocotyledoneae | Cardoso |
| *Psychotria nuda* | Rubiaceae | Gentianales | Asteridae | Cardoso |
| *Psychotria sp 1* | Rubiaceae | Gentianales | Asteridae | Cardoso |
| *Psychotria sp 2* | Rubiaceae | Gentianales | Asteridae | Cardoso |

Outliers were defined as fruit species with zero fruit color density.

*Orders to which the outlier species belong were not included in the order-level phylogenetic conservatism analysis because of low sampling (< 10 species per order).

†*Elaeocarpus angustifolius* fruit color was acquired from the published fruit reflectance spectrum in Lee (1991).

**Table 4** Results of Blomberg's $K$ test for phylogenetic signal in fruit color variables

| Color coordinate | $K$ | Observed PIC variance | Mean random PIC variance | $P$ value |
|---|---|---|---|---|
| $x$ | 0.1667 | 0.0070 | 0.0093 | 0.001 |
| $y$ | 0.1596 | 0.0024 | 0.0030 | 0.001 |
| $z$ | 0.1613 | 0.0032 | 0.0040 | 0.001 |

$x$, $y$ and $z$ correspond to the coordinates of each color in the avian sensory space and are treated as traits in the analysis. PIC, phylogenetic independent contrast.

To understand the effect of outliers on global fruit gamut diversity, we performed all analyses of the null model, including the calculation of the random pool, again after removal of the five outliers from the fruit gamut (truncated gamut). The results did not change qualitatively compared with the first null model for any of the localities, except for Cardoso (Table 2).

### Phylogenetic conservatism

Fruit color viewed in the avian sensory space showed significantly very low phylogenetic signal (Table 4). This implies that the evolution of fruit color overall has not been greatly 'constrained' by phylogenetic relatedness.

We also found no evidence for order-level effects on fruit color diversity. Of the 18 orders, 16 showed color volumes not significantly different from those expected by chance, whereas two orders (Laurales, Solanales) had volumes marginally smaller than expected (Table 5). Overall, however, orders matched expected random color diversities. Variation in the composition of orders within localities is thus not likely to affect fruit color diversity of localities.

In addition, the volumes of the four major angiosperm clades (Rosidae, Asteridae, Monocotyledons and Magnoliidae) overlapped substantially, indicating that these clades independently populated the same regions of the color space and entailed a high level of homoplasy in fruit color (Fig. 5, Table 6). Importantly, the percentage overlap between the clade volumes did not differ from the overlap between random clades (Table 6, all $P > 0.05$). In other words, reshuffling the phylogenetic relationships results in the same clade overlap as observed, indicating that, at this level, phylogeny does not have an effect on fruit color diversity.

**Table 5** Phylogenetic conservatism analysis comparing volumes of existing order-level clades with simulated orders

| Order name | Color volume | 95% CI | $n$ |
|---|---|---|---|
| Apiales | 0.0016 | 0.0007–0.0070 | 19 |
| Aquifoliales | 0.0011 | 0.0002–0.0043 | 12 |
| Arecales | 0.0022 | 0.0018–0.0112 | 36 |
| Cornales | 0.0025 | 0.0002–0.0040 | 11 |
| Dipsacales | 0.0016 | 0.0008–0.0077 | 21 |
| Ericales | 0.0058 | 0.0041–0.0176 | 79 |
| Fabales | 0.0013 | 0.0005–0.0061 | 17 |
| Gentianales | 0.0123 | 0.0052–0.0210 | 106 |
| Lamiales | 0.0059 | 0.0008–0.0071 | 20 |
| Laurales | *0.0013* | 0.0013–0.0094 | 28 |
| Magnoliales | 0.0018 | 0.0012–0.0091 | 27 |
| Malpighiales | 0.0038 | 0.0034–0.0162 | 65 |
| Myrtales | 0.0051 | 0.0062–0.0236 | 133 |
| Ranunculales | 0.0023 | 0.0004–0.0055 | 15 |
| Rosales | 0.0067 | 0.0054–0.0215 | 113 |
| Santalales | 0.0022 | 0.0011–0.0087 | 25 |
| Sapindales | 0.0027 | 0.0017–0.0110 | 34 |
| Solanales | *0.0017* | 0.0017–0.0110 | 34 |

Numbers in italics indicate marginally smaller color volumes of orders than expected by the simulation. Other numbers indicate no difference between observed and simulated order color volume. $n$, number of species in each order (only orders with > 10 species were used in the analysis).

The composite phylogenetic tree used in our analyses can be found in Supporting Information Fig. S1.

## Discussion

Analysis of the fruit color gamut diversity revealed that, as predicted, fruits occupy a restricted area in the avian sensory space. The fruit color gamut showed a significantly smaller diversity than the flower gamut, supporting the Whitney hypothesis. Moreover, in line with the prediction from the similarity hypothesis, regions as well as communities showed smaller volumes than expected by chance inside the occupied color space area. Interestingly, five outlier species with distinct colors contributed disproportionally to the overall fruit gamut diversity. Lastly, we found very low phylogenetic signal in fruit color parameters derived from the color space. Moreover, fruit color diversity was not affected significantly by membership in orders, and major clades showed a high degree of homoplasy. Together, these results refute the phylogenetic conservatism hypothesis at these levels.

## Why most of the color space is empty

As predicted, the color gamut of bird-dispersed fruits occupied only a fraction (*c.* 17%) of the avian UVS sensory space, leaving *c.* 83% of it empty. Studies of morphospace commonly find that only a subset of the available space is filled by existing forms. As such, our finding is, at first glance, not surprising. Nevertheless, the question of why fruits leave such a seemingly vast area unexplored remains unanswered. To approach this question, we use the reasons for emptiness in morphospace, introduced in the Introduction: constraints do not allow the existence of some forms; selection is acting against some forms; some forms simply have not (yet) evolved; or some forms are impossible to produce in nature (McGhee, 1999).

Natural colors do not seem to be able to fill out the avian color space. Even though it is almost twice as diverse as the fruit gamut volume, the flower gamut volume was also restricted to *c.* 30% of the color space. Moreover, bird plumage colors fill out roughly the same limited volume as a large set of plant colors (Stoddard & Prum, 2011). Indeed, to fill out the color space, plants and birds would have to produce highly saturated colors that predominantly stimulate only one or two cone types in the bird's eye. Such colors result only from monochromatic light or spectra with a steep increase in reflectance within a very narrow bandwidth of wavelengths. Yet, natural colors are characterized by broad and smooth reflectance curves that necessarily stimulate more than two cone types. Therefore, some of the colors of the avian sensory morphospace are not feasible in nature (Vorobyev, 2003; Stoddard & Prum, 2011).

Chemical constraints of the color-producing pigments may also contribute to the areas in the color space left empty by fruit and flower colors. In bird plumages, each type of utilized pigment only accounts for a small amount of the overall plumage color diversity (Stoddard & Prum, 2011). This is not very surprising as classes of pigments are empirically known to produce only specific sets of colors. Carotenoids, for example, typically produce yellow, orange and red colors according to human vision (Goodwin, 1976). It is very likely that the limited color diversity produced by each pigment type results from its chemical light absorption properties, which allow the molecule to react only to specific wavelengths. Such chemical constraints are intrinsic properties of the specific color production mechanism and will contribute to the boundaries of the observed fruit and flower color diversity.

## Fruit colors are less diverse than flower colors

Given that not all of the avian sensory space can be filled, the next question that arises is: has evolution not yet produced some of the feasible colors in fruits, or is selection acting against particular colors? Comparison with the flower color gamut allows us to approach this question. Although it is not entirely clear when flowers and fruits first evolved (e.g. see Smith *et al.*, 2010), we know that they diversified greatly starting in the mid-Cretaceous (Friis *et al.*, 2006) and have had a long time to diversify their colors. Moreover, fruits originate developmentally from flowers and presumably share similar physiological and biochemical constraints. Thus, *ceteris paribus*, fruits and flowers should be able to produce the same color diversity. However, we showed that flowers have achieved roughly the two-fold diversity of fruits in the avian receptor space, supporting the Whitney hypothesis. Importantly, *c.* 93% of the fruit volume overlaps with the flower volume, but only *c.* 50% of the flower volume overlaps with the fruit volume, which means that the fruit color volume is positioned inside the flower color volume. The volume rarefaction analysis for the fruit and the flower gamut also supports the Whitney hypothesis. Although sampling for both gamuts is incomplete, the statistically significant difference in the slope of the curves shows that, overall, flowers achieve higher color diversity than fruits. These results agree with Whitney (2009), despite the fact that, in that study, fruit and flower color was coded in RGB values inferred from human color descriptions in the literature and that diversity was analyzed in a combined color and size morphospace. Thus, fruits do not achieve the color diversity shown by flowers, although evolutionary time and similar constraints suggest that they should be able to.

The difference in color diversity of fruits and flowers may result from the different types of selective pressures acting on them (Schaefer *et al.*, 2004; Whitney, 2009). In many flowers, selection for color diversification between species is strong because conspecific flowers of dioecious plants and plants separating the timing of male and female function need to be visited at least twice by the same insect for successful pollination. Flower colors (and morphology) of different species are therefore selected to be distinct and memorable to pollinators (Chittka *et al.*, 1999; Gumbert *et al.*, 1999; Chittka & Schürkens, 2001). By contrast, most plants probably do not profit more from tight associations with seed dispersers as this leads to an increased co-deposition of conspecific seeds, which can decrease seedling recruitment (Harms *et al.*, 2000; Russo & Augspurger, 2004). Accordingly, although flowers are expected to profit from having distinct, recognizable colors, fruits may tend to converge to similar colors to increase the number of dispersers. This is supported by the finding that, although flower pollinator networks might actually be less specialized than previously thought (e.g. Herrera, 1996, 2005; Waser *et al.*, 1996), they nevertheless show a higher degree of specialization than fruit disperser networks (Blüthgen *et al.*, 2007). Thus, different selection regimes on fruit and flower colors may explain why some colors have not evolved in fruits, although they have evolved in flowers.

Another possible source of diverse selective pressures on fruits and flowers lies in the multiple functional roles of pigments in plants (reviewed in Gould, 2004; Cazzonelli, 2011). Carotenoids function as accessory pigments to increase the efficiency of photosynthesis within chloroplasts, and both anthocyanins and carotenoids function in the photoprotection of sensitive tissues (Young, 1991; Smillie & Hetherington, 1999; Hormaetxe *et al.*, 2004). Moreover, anthocyanins have been associated with tolerance to abiotic stressors, such as heat or drought (Strauss & Whittall, 2006), inhibit the growth of fruit-rotting fungi (Schaefer *et al.*, 2008) and function as strong antioxidants that improve immune response in birds (Catoni *et al.*, 2008). The multiple roles of pigments suggest that multiple selective pressures, not only from mutualists, but also from pathogens and from the abiotic

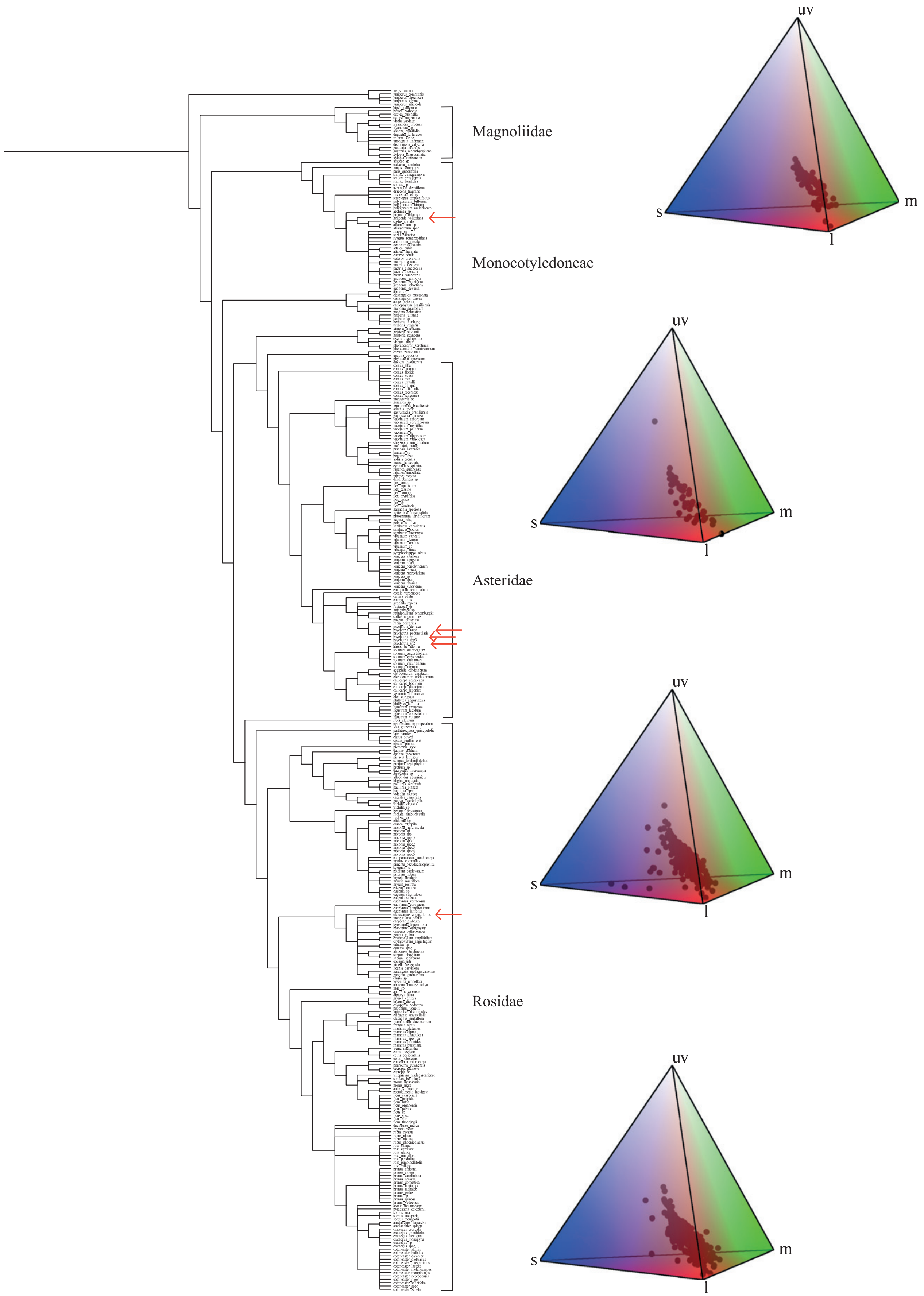
uv
s
m
l
Magnoliidae
Monocotyledoneae
uv
s
m
l
Asteridae
uv
s
m
l
Rosidae
uv
s
m
l

**Table 6** Results of phylogenetic conservatism analysis showing pairwise percentage color volume overlap of four major clades

| | Asteridae | Rosidae | Monocotyledoneae | Magnoliidae | *n* | Color volume |
|---|---|---|---|---|---|---|
| Asteridae | | 69.7 (49.9–98.2) | 28.6 (13.4–66.1) | 17.7 (15.3–70.9) | 328 | 0.0238 |
| Rosidae | 84.2 (41.6–95.2) | | 34.7 (11.6–62.5) | 21.5 (14.1–68.3) | 423 | 0.0197 |
| Monocotyledoneae | 82.6 (64.5–99.6) | 82.8 (70.1–99.7) | | 40.3 (31.8–97.4) | 64 | 0.0083 |
| Magnoliidae | 99.1 (63.2–99.6) | 99.4 (68.0–99.6) | 78.3 (23.0–92.1) | | 79 | 0.0043 |

All numbers in the first four columns are percentage values. Single values refer to pairwise percentage overlap and values in brackets indicate the 95% CI of the percentage overlap between simulated clades. Percentage overlap values, as well as CI ranges, are calculated relative to the clade of the respective row. The table should be read in the following way: '84.2% of the volume of Rosidae overlaps with the volume of Asteridae, and 69.7% of the volume of Asteridae overlaps with the volume of Rosidae'.

environment, act on pigments (Catoni *et al.*, 2008; Schaefer *et al.*, 2008). However, such multiple selective pressures on color might be less pronounced in flowers relative to fruits, because color advertisement in flowers is spatially decoupled from reward (Schaefer *et al.*, 2004). Specifically, dispersers, by consuming the fruit, also consume the signaling structure containing the pigments, namely the fruit epidermis, but pollinators do not consume flower petals in addition to the reward. Thus, pigments coloring flowers cannot function to protect the rewarding nutrients from antagonists, nor can they function as an additional reward to pollinators in the form of antioxidants in the way in which pigments in fruits can. The possible presence of conflicting selective pressures on pigments in fruits and their likely absence in flowers may further contribute to the observed smaller color diversity of fruits relative to flowers.

We report in this study that flower colors are roughly twice as diverse as fruit colors. It should be noted that this number is tied to the type of sensory space used to quantify diversity. The avian color space is easily recognizable as the appropriate color space for the predominantly bird-dispersed fruits. However, the choice of a morphospace for flower color is less straightforward. As the flower gamut consists of species pollinated by different classes of animals (bees, butterflies, etc.) with different visual systems, their higher diversity (relative to fruits) may reflect a more distinct disperser assemblage. At the same time, our analyses based on the avian eye may not accurately reflect the flower color diversity perceived by pollinators. Thus, naturally, the perceived color diversity will differ between organisms. Yet, birds with their tetrachromatic visual system are expected to possess higher color discrimination ability than, for example, bees, which are trichromats. Although color diversity is a relative figure depending on the visual system, the diversity of fruits and flowers reported here is based on a visual system that offers high color discrimination.

## Local fruit color diversity

Overall, the diversities and color distributions of localities differed significantly from the expectations of the locality null model. All but one of the localities (Cardoso, Brazil) occupied limited areas in the gamut volume. Although fruit colors were distributed more evenly in space than randomly expected in the Peruvian communities, the three regions as well as Cardoso showed a higher heterogeneity in the spatial positions of fruit colors.

What is leading to the limited color diversity in 14 of the localities? Although the localities may differ in their phylogenetic composition, the significantly low phylogenetic signal in fruit color, as well as the lack of clade-level effects, strongly indicates that limited fruit color diversity is not a strictly phylogenetic phenomenon. The low fruit color diversity of most localities therefore seems to support the similarity hypothesis. Because plants probably do not profit from tight associations with seed dispersers, fruit colors may not face strong diversification, but rather become more similar to those signals that are both relatively easy to produce and detectable against the foliage background for birds (Schaefer *et al.*, 2004).

Alternatively, not all colors inside the fruit gamut may be feasible in fruits, as our null model assumes. Indeed, the drastic reduction in the fruit gamut volume after removal of the five outlier species shows that there are gaps of unoccupied color space within the fruit gamut volume. Such gaps point to the discontinuous nature of fruit color, and theoretically contribute to the explanation of the limited color diversity seen in localities. In practice, however, the analysis with the truncated gamut shows that at least the holes generated by the five outliers do not affect qualitatively the results, although the discrepancy between observed and random diversity decreases.

The presence of fruit gamut gaps is intriguing. Forty of the 84 unique flower colors (i.e. no fruit colors in close proximity) were found inside the fruit gamut, which means that they lie within gaps in the fruit gamut. Some of the gaps may thus not be caused by an inability of plants to produce these colors, as flowers can apparently produce them. Therefore, the global pattern of differences in color diversities of the fruit and flower gamut also repeats itself inside the boundaries of the gamuts. Color similarity in fruits and limitations arising from the multiple roles and selective pressures on fruit color presumably shape gaps in the fruit gamut.

Some of the gaps in the fruit gamut are defined by outliers, species whose colors, by definition, differ from all other fruit

**Fig. 5** Composite phylogenetic tree of the fruit gamut with the four major clades depicted in the avian sensory space. The five outlier species are indicated with red arrows. For visualization purposes, a phylogenetic tree of only a subset of the species (from the following localities: Germany, Spain, Florida, Pantanal, Kakamega Forest, Amazonian Forest and Cardoso) is shown. Relative cone catches uv, s, m and l are maximized at the respective vertex of the tetrahedron. The color of the tetrahedron is a crude approximation for illustration purposes.

colors. The existence of outlier species documents that some lineages have apparently evolved the ability to evade the limitations acting on the majority of fruit colors. For example, one outlier species is *E. angustifolius*, one of three plant species known to produce its fruit color not through pigments, but by using a special light-reflecting nanostructure in the fruit epidermis (Lee, 1991; Lee *et al.*, 2000; Vignolini *et al.*, 2012). Notably, similar structural color mechanisms are probably more widespread in flowers than fruits (Glover & Whitney, 2010). Stoddard & Prum (2011) showed that, in birds, plumage color diversity has been largely increased by the evolution of structural mechanisms which produce colors that pigments would apparently be unable to create. Similarly, structural colors in plants could allow flowers and fruits, such as *E. angustifolius*, to overcome the existing chemical constraints imposed by anthocyanin and carotenoid pigment colors. Indeed, the more widespread occurrence of structural colors in flowers could be a further proximate explanation for their higher color diversity relative to fruits.

### Conclusion and outlook

Fruit colors occupy only *c.* 17% of the available avian UVS sensory color space, leaving vast areas of color unexplored. We discuss both proximate and ultimate factors that may explain this pattern. We note that some areas of the color space cannot be reached by natural, smooth spectra, and chemical constraints inherent to plant pigments probably further limit fruit color diversity. Moreover, we show that fruit colors have achieved only roughly half the color diversity exhibited by flowers in the avian UVS color space. Different types of selection on fruit and flower colors, as well as possibly conflicting selective pressures on fruit pigments, may explain this difference. The global pattern of limited fruit color diversity extends down to the local level. Fruit colors of localities are more similar and less diverse than expected by chance, but this pattern does not seem to result from phylogenetic conservatism, as the studied clades show extensive homoplasy. Thus, the *c.* 83% of the avian UVS color space that fruits leave empty can be explained, at least in part, by a combination of impossible colors, chemical constraints of pigments, multiple, probably conflicting, selective pressures on those pigments and selection that possibly favors similar colors in fruits of the same locality.

A range of questions regarding fruit color evolution remain unanswered. For example, is the similarity in fruit colors found in localities the result of convergent evolution, or could it be the result of other processes, such as ecological sorting? Further, what advantages do distinct fruit colors produced by novel mechanisms convey? Studying fruit color in the light of biological market theory (Noë & Hammerstein, 1994), which assumes that 'producers' (plants) compete for the attention of 'consumers' (seed dispersers), and that fruit color functions as an advertisement to consumers, and predicts the evolution of distinct fruit colors that are coupled to high reward, could provide a useful approach to this question.

## Acknowledgements

K.E.S. acknowledges funding by the Volkswagen Foundation and the German Academic Exchange Service (DAAD). K.B.G., D.M.D. and M.S. acknowledge support from the research funding program 'LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz' of Hessen's Ministry of Higher Education, Research, and the Arts. Field work in Peru was also supported partly by a grant from the German Academic Exchange Service to D.M.D.

## References

**APG III. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.

**Arnold SEJ, Faruq S, Savolainen V, McOwan PW, Chittka L. 2010.** FReD: the floral reflectance database – a web portal for analyses of flower colour. *PLoS ONE* **5**: 1–9.

**Blomberg SP, Garland T Jr., Ives AR. 2003.** Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

**Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N. 2007.** Specialization, constraints, and conflicting interests in mutualistic networks. *Current Biology* **17**: 341–346.

**Bowmaker JK, Heath LA, Wilkie SE, Hunt DM. 1997.** Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research* **37**: 2183–2194.

**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.

**Cantino PD, Doyle JA, Graham SW, Judd WS, Olmstead RG, Soltis DE, Soltis PS, Donoghue MJ. 2007.** Towards a phylogenetic nomenclature of Tracheophyta. *Taxon* **56**: 822–846.

**Catoni C, Schaefer HM, Peters A. 2008.** Fruit for health: the effect of flavonoids on humoral immune response and food selection in a frugivorous bird. *Functional Ecology* **22**: 649–654.

**Cazzonelli CI. 2011.** Carotenoids in nature: insights from plants and beyond. *Functional Plant Biology* **38**: 833–847.

**Chittka L, Schürkens S. 2001.** Successful invasion of a floral market. *Nature* **411**: 653.

**Chittka L, Thomson JD, Waser NM. 1999.** Flower constancy, insect psychology, and plant evolution. *Naturwissenschaften* **86**: 361–377.

**Donatti CI, Galetti M, Pizo MA, Guimarães PR, Jordano P. 2007.** Living in the land of ghosts: fruit traits and the importance of large mammals as seed dispersers in the Pantanal. In: Dennis AJ, Schupp EW, Green RA, Westcott DA, eds. *Seed dispersal: theory and its implications in a changing world.* Wallingford, UK: Commonwealth Agricultural Bureau International, 104–123.

**Endler JA, Mielke PW Jr. 2005.** Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society* **86**: 405–431.

**Endler JA, Westcott DA, Madden JR, Robson T. 2005.** Animal visual signals and the evolution of color patterns: sensory processing illuminates signal evolution. *Evolution* **59**: 1795–1818.

**Fischer KE, Chapman CA. 1993.** Frugivores and fruit syndromes: differences in patterns at the genus and species level. *Oikos* **66**: 472–482.

**Flörchinger M, Braun J, Böhning-Gaese K, Schaefer HM. 2010.** Fruit size, crop mass, and plant height explain differential fruit choice of primates and birds. *Oecologia* **164**: 151–161.

**Friis EM, Pedersen KR, Crane PR. 2006.** Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. *Palaeogeography, Palaeoclimatology, Palaeoecology* **232**: 251–293.

**Glover BJ, Whitney HM. 2010.** Structural colour and iridescence in plants: the poorly studied relations of pigment colour. *Annals of Botany* **105**: 505–511.

**Goldsmith TH. 1990.** Optimization, constraint, and history in the evolution of eyes. *Quarterly Review of Biology* **65**: 281–322.

**Goodwin TW. 1976.** Distribution of carotenoids. In: Goodwin TW, ed. *Chemistry and biochemistry of plant pigments.* New York, NY, USA: Academic Press, 225–261.

**Gould KS. 2004.** Nature's Swiss army knife: the diverse protective roles of anthocyanins in leaves. *Journal of Biomedicine & Biotechnology* **2004**: 314–320.

**Gumbert A, Kunze J, Chittka L. 1999.** Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society B, Biological Sciences* **266**: 1711–1716.

**Harms KE, Wright SJ, Calderón O, Hernández A, Herre EA. 2000.** Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature* **404**: 493–495.

**Hart NS, Hunt DM. 2007.** Avian visual pigments: characteristics, spectral tuning, and evolution. *American Naturalist* **169**: 7–26.

**Herrera CM. 1984.** Adaptation to frugivory of Mediterranean avian seed dispersers. *Ecology* **65**: 609–617.

**Herrera CM. 1996.** Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: Lloyd DG, Barrett SCH, eds. *Floral biology*. New York, NY, USA: Chapman and Hall, 65–87.

**Herrera CM. 2005.** Plant generalization on pollinators: species property or local phenomenon? *American Journal of Botany* **92**: 13–20.

**Hormaetxe K, Hernández A, Becerril JM, García-Plazaola JI. 2004.** Role of red carotenoids in photoprotection during winter acclimation in *Buxus sempervirens* leaves. *Plant Biology* **6**: 325–332.

**Janson CH. 1983.** Adaptation of fruit morphology to dispersal agents in a neotropical forest. *Science* **219**: 187–189.

**Jordano P. 2000.** Fruits and frugivory. In: Fenner M, ed. *Seeds: the ecology of regeneration in plant communities*. Wallingford, UK: CAB International, 125–165.

**Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO. 2010.** Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**: 1463–1464.

**Lee DW. 1991.** Ultrastructural basis and function of iridescent blue colour of fruits in Elaeocarpus. *Nature* **349**: 260–262.

**Lee DW, Taylor GT, Irvine AK. 2000.** Structural fruit coloration in *Delarbrea michieana* (Araliaceae). *International Journal of Plant Sciences* **161**: 297–300.

**Lomáscolo SB, Levey DJ, Kimball RT, Bolker BM, Alborn HT. 2010.** Dispersers shape fruit diversity in *Ficus* (Moraceae). *Proceedings of the National Academy of Sciences* **107**: 14668–14672.

**Lomáscolo SB, Schaefer HM. 2010.** Signal convergence in fruits: a result of selection by frugivores? *Journal of Evolutionary Biology* **23**: 614–624.

**McGhee GR. 1999.** *Theoretical morphology: the concept and its applications*. New York, NY, USA: Columbia University Press.

**McGhee GR. 2007.** *The geometry of evolution: adaptive landscapes and theoretical morphospaces*. New York, NY, USA: Cambridge University Press.

**Noë R, Hammerstein P. 1994.** Biological markets: supply and demand determine the effect and mating mutualism of partner choice in cooperation, mutualism and mating. *Behavioral Ecology and Sociobiology* **35**: 1–11.

**Osorio D, Vorobyev M. 2008.** A review of the evolution of animal colour vision and visual communication signals. *Vision Research* **48**: 2042–2051.

**Patterson BD, Stotz DF, Solari S, Fitzpatrick JW, Pacheco V. 1998.** Contrasting patterns of elevational zonation for birds and mammals in the Andes of southeastern Peru. *Journal of Biogeography* **25**: 593–607.

**Russo SE, Augspurger CK. 2004.** Aggregated seed dispersal by spider monkeys limits recruitment to clumped patterns in *Virola calophylla*. *Ecology Letters* **7**: 1058–1067.

**Sanders HL. 1968.** Marine benthic diversity: a comparative study. *American Naturalist* **102**: 243–282.

**Schaefer HM, Rentzsch M, Breuer M. 2008.** Anthocyanins reduce fungal growth in fruits. *Natural Product Communications* **3**: 1267–1272.

**Schaefer HM, Ruxton GD. 2011.** *Plant–animal communication*. New York, NY, USA: Oxford University Press.

**Schaefer HM, Schaefer V, Levey DJ. 2004.** How plant–animal interactions signal new insights in communication. *Trends in Ecology & Evolution* **19**: 577–584.

**Schaefer HM, Schaefer V, Vorobyev M. 2007.** Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *American Naturalist* **169**: 159–169.

**Schaefer HM, Schmidt V. 2004.** Detectability and content as opposing signal characteristics in fruits. *Proceedings of the Royal Society B, Biological Sciences* **271**: 370–373.

**Schmidt V, Schaefer HM, Winkler H. 2004.** Conspicuousness, not colour as foraging cue in plant–animal signalling. *Oikos* **106**: 551–557.

**Smillie RM, Hetherington SE. 1999.** Photoabatement by anthocyanin shields photosynthetic systems from light stress. *Photosynthetica* **36**: 451–463.

**Smith SA, Beaulieu JM, Donoghue MJ. 2010.** An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences, USA* **107**: 5897–5902.

**Snow DW. 1971.** Evolutionary aspects of fruit-eating by birds. *Ibis* **113**: 194–202.

**Stoddard MC, Prum RO. 2008.** Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of new world buntings. *American Naturalist* **171**: 755–776.

**Stoddard MC, Prum RO. 2011.** How colorful are birds? Evolution of the avian plumage color gamut. *Behavioral Ecology* **22**: 1042–1052.

**Stoddard MC, Stevens M. 2011.** Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution* **65**: 2004–2013.

**Strauss SY, Whittall JB. 2006.** Non-pollinator agents of selection on floral traits. In: Harder LD, Barrett SCH, eds. *Ecology and evolution of flowers*. New York, NY, USA: Oxford University Press, 120–135.

**Sumner P, Mollon JD. 2003.** Colors of primate pelage and skin: objective assessment of conspicuousness. *American Journal of Primatology* **59**: 67–91.

**Valido A, Schaefer HM, Jordano P. 2011.** Colour, design and reward: phenotypic integration of fleshy fruit displays. *Journal of Evolutionary Biology* **24**: 751–760.

**Vignolini S, Rudall PJ, Rowland AV, Reed A, Moyroud E, Faden RB, Baumberg JJ, Glover BJ, Steiner U. 2012.** Pointillist structural color in *Pollia* fruit. *Proceedings of the National Academy of Sciences, USA* **109**: 15712–15715.

**Vorobyev M. 2003.** Coloured oil droplets enhance colour discrimination. *Proceedings of the Royal Society B, Biological Sciences* **270**: 1255–1261.

**Vorobyev M, Osorio D. 1998.** Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B, Biological Sciences* **265**: 351–358.

**Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996.** Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043–1060.

**Webb CO, Ackerly DD, Kembel SW. 2008.** Phylocom: software for the analysis of phylogenetic community structure and trait evolution. *Bioinformatics* **24**: 2098–2100.

**Webb CO, Donoghue MJ. 2005.** Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* **5**: 181–183.

**Wheelwright NT. 1988.** Fruit-eating birds and bird-dispersed plants in the tropics and temperate zone. *Trends in Ecology & Evolution* **3**: 270–274.

**Wheelwright NT, Janson CH. 1985.** Colors of fruit displays of bird-dispersed plants in two tropical forests. *American Naturalist* **126**: 777–799.

**Whitney KD. 2009.** Comparative evolution of flower and fruit morphology. *Proceedings of the Royal Society B, Biological Sciences* **276**: 2941–2947.

**Willson MF, Irvine AK, Walsh NG. 1989.** Geographic comparisons in some Australian vertebrate dispersal syndromes and New Zealand plant communities, with geographic comparisons. *Biotropica* **21**: 133–147.

**Willson MF, Whelan CJ. 1990.** The evolution of fruit color in fleshy-fruited plants. *American Naturalist* **136**: 790–809.

**Young AJ. 1991.** The photoprotective role of carotenoids in higher plants. *Physiologia Plantarum* **83**: 702–708.

## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Composite phylogeny of plant species of the fruit color gamut.

**Methods S1** Details on geographic location and size of localities, and prevailing vegetation type.

**Video S1** Fruit color gamut viewed in the rotating avian sensory space.