

Fruit colour conceals endocarp dimorphism from avian seed dispersers in a tropical beach plant, *Scaevola taccada* (Goodeniaceae), found in Okinawa

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Abstract: Theory predicts that honest signalling strategies will not always be evolutionarily stable in interspecific communication, yet to demonstrate such a transition of signalling modality between honesty and dishonesty in the wild would be difficult. An endocarp dimorphism has been found in *Scaevola taccada* fruits: a morph with a cork substrate that facilitates ocean current seed dispersal and a morph without the cork. Both types of fruit are covered with sugar-containing flesh, and are similar in size and colour to one another (at least from a human perspective). The apparent lack of external differences between morphotypes could potentially degrade mutualistic relations between the plant and seed-dispersing birds because the presence of a cork could lower the fruit's nutritional value. Thus, unless seed dispersers can discriminate between the different types of fruit, this system may provide an example of a transition between honest and dishonest signalling. We examined *S. taccada* fruit and leaf colours from an avian visual perspective. Even though the fruits and leaves were different in colour from one another to birds, there was no perceivable difference in the colours between fruit morphotypes. Therefore, fruit colour is not an honest indicator of reward to seed dispersers. Further, we propose an adoption of a statistical method in avian visual modelling studies that avoids the common statistical errors, such as violation of the congruence principle.

Key Words: avian seed dispersal, avian visual model, cryptic dimorphism, deception, interspecific communication

INTRODUCTION

Biological communication signals can be mutualistic or parasitic, depending on whether the receivers can gain benefits by responding to the signals (honest signalling) or the signals exist at the expense of the receiver (deceptive or dishonest signalling) (Foster & Delay 1998, Galetti 2002, Stuart-Fox 2005). However, theory predicts that both honest and dishonest signalling strategies will not always be evolutionarily stable in interspecific communication (Freckleton & Côté 2003). Thus, mutualists can become parasitic and vice versa, even in the context of plant–animal interactions: an individual plant may reduce the level of reward to conserve resources without indicating the change in rewards to mutualistic agents. Despite the theoretical prediction, it would be difficult to demonstrate such signalling bimodality in wild animal–plant systems because the transition from mutualistic to parasitic state

is expected to be subtle and multimodal (Edwards & Yu 2007).

Scaevola taccada is a widely distributing tropical beach plant, whose fruits have an endocarp composed of a cork substrate around the seed, facilitating flotation (Emura *et al.* 2014). It is likely that *S. taccada* seeds are dispersed by ocean currents as well as by frugivorous birds (Emura *et al.* 2012, Howarth *et al.* 2003). However, Emura *et al.* (2014) discovered a cryptic dimorphism in the endocarp of this plant: although most plants bear fruit with the cork endocarp (C+), fruits of some individuals consistently lack such a structure (C–), even though both types of fruit have sugar-containing flesh around the seed. It is probable that these morphotypes have adapted alternatively: plants with the C+ fruit disperse seeds broadly through ocean currents (along with avian dispersal), while those with the C– fruit remain relatively localized through avian dispersal.

Despite the difference in assumed advantages of the endocarp types, the fruit colour seems not to differ between them, at least in the human vision (Emura *et al.* 2014).

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The lack of colour differentiation indicates that the fruit colour of C+ plants might relatively be a dishonest signal because of the apparently reduced nutritional contents and increased indigestible substrates. However, whether the fruit colour is actually indiscernible to seed dispersers is unclear, since birds have higher sensitivity to colours than mammals including humans (Endler & Mielke 2005, Tanaka *in press*, Vorobyev & Osorio 1998). Therefore, the fruit colour in bird vision must be examined to determine whether seed-dispersing birds can distinguish between the colours of C+ fruits and C- fruits.

We focused on the dissimilarity in colour between fruits and leaves, as well as fruits of *S. taccada* morphotypes. The contrast in colour between fruits and leaves should be a key attractant for seed-dispersing birds, because perceived colour of an object is in principle its contrast against the background colour (Koshitaka *et al.* 2008, Osorio & Vorobyev 1996, Vorobyev & Osorio 1998). To avoid C+ fruits, seed-dispersing birds should memorize the fruit colour against the leaf background (i.e. fruit-leaf contrast) because the endocarp morphotypes are consistent within individual plants, and birds should move to another plant to encounter fruits of the C- morphotype. Therefore, great dissimilarity in fruit-leaf contrast between morphotypes is essential for *S. taccada* to establish an honest signal. We hypothesized that the fruit colour of *S. taccada* will be a dishonest signal if the colour contrasts of fruits against the leaf background are indistinguishable between morphotypes in the vision of potential seed-dispersing birds, and tested it through the avian visual model.

MATERIALS AND METHODS

Study species

Scaevola taccada (Gaertn.) Roxb. is a coastal shrub, distributing widely in tropical and subtropical Indo-Pacific coastal lands, from East Africa to Oceania and Japan. The cork substrate of the endocarp facilitates flotation, as fruits with the cork endocarp float for more than 6 mo while fruits without cork sink relatively rapidly, i.e. within 1 wk (Emura *et al.* 2014). The endocarp dimorphism might serve as an alternative strategy of seed dispersal within the same species, since a considerable rate of gene flow between individuals of the two morphs has been detected (Emura *et al.*, unpubl. data).

The size of the indigestible part of C+ fruits is 6.61 mm in diameter, whereas the one for C- is 3.93 mm due to the lack of cork endocarp (Emura *et al.* 2014). This size difference results not only in a 10% reduction in the volume of digestive pulp in C+ fruits, but also in an almost five times greater volume of the indigestible part of C+ than that of C-, 0.15 ml for C+ and 0.031 ml for C-,

assuming spherical shapes (i.e. $\frac{4}{3}\pi r^3$ where $r = \frac{\text{diameter}}{2}$). Moreover, the pulp of C+ fruits contains more water than that of C- (Emura *et al.*, unpubl. data), suggesting a much lower nutritional reward to seed dispersers than the one expected by the structural difference between morphotypes. Therefore, the total amount of reward a C+ fruit provides to birds should be less than that of the C- type, and simultaneously, the amount of indigestible substrate should be greater despite the lack of difference in external sizes (Emura *et al.* 2014).

Three fruits and leaves of *S. taccada* were collected from each of 49 individuals (C+: C-, $N = 24: 25$) in four adjacent sites: Cape Zanpa (26°07'05"N, 127°12'14"E), $N = 30$ plants (C+: C- = 5: 25); Ikei Island (26°06'07"N, 127°16'08"E), $N = 12$ (C+: C- = 12: 0); Coast on Kim Bay (26°06'09"N, 127°15'01"E), $N = 4$ (C+: C- = 4: 0); Yabuchi Island (26°05'06"N, 127°15'05"E), $N = 3$ (C+: C- = 3: 0), all located on the main island of Okinawa, Japan. The total numbers of fruits and leaves were both $n = 147$. To avoid decaying, after these samples were collected in August 2014, they were maintained at ambient temperatures, transported by air to Tokyo, and measured, all within several days.

Measurement

Reflectance spectra of fruits and leaves were measured using a spectrophotometer (Jaz-EL-200, Ocean Optics, Dunedin, FL, USA) with light irradiated by a deuterium-tungsten-halogen light source (DT-MINI-2-GS, Ocean Optics, Dunedin, FL, USA). Before and during measurements, the spectrometer was calibrated (light/dark) using a diffuse reflectance standard (WS-1, Ocean Optics, Dunedin, FL, USA). Fruits and leaves were placed in a black box during measurements. We repeated measurements three times for each fruit/leaf, in different sections for each measurement ($n = 441$). The total number of measured reflectance spectra was $n = 882$.

We first calculated photon captures Q_i by the ultraviolet-, short-, medium- and long-wavelength-sensitive single-cones (or UVS, SWS, MWS and LWS, respectively), as well as by the double-cone, with the measured reflectance $R(\lambda)$ and photoreceptor sensitivity $C_i(\lambda)$,

$$Q_i = \int_{300}^{700} R(\lambda)C_i(\lambda).$$

The single- and double-cone sensitivity of the blue tit *Cyanistes caeruleus* (Hart *et al.* 2000) with its cone abundance (Hart 2001) were adopted because observed seed dispersers of the plant are the blue rock-thrush *Monticola solitarius* (Emura *et al.* 2012) and the brown-eared bulbul *Hypsipetes amaurotis stejnegeri* (Kawakami

et al. 2009), both of which are classified as Passerida, and thus, likely to have the UVS (ultraviolet sensitive) vision (Ödeen *et al.* 2011).

We then calculated the JND (just noticeable difference), a discrimination threshold between a given pair of colours, from their photon captures based on the Vorobyev–Osorio model (Vorobyev & Osorio 1998): JNDs were obtained from

$$\text{JND} = \sqrt{\Delta S} = \sqrt{\frac{(\omega_{UV}\omega_S)^2(\Delta f_L - \Delta f_M)^2 + (\omega_{UV}\omega_M)^2(\Delta f_L - \Delta f_S)^2 + (\omega_{UV}\omega_L)^2(\Delta f_M - \Delta f_S)^2 + (\omega_S\omega_M)^2(\Delta f_L - \Delta f_{UV})^2 + (\omega_S\omega_L)^2(\Delta f_M - \Delta f_{UV})^2 + (\omega_M\omega_L)^2(\Delta f_S - \Delta f_{UV})^2}{(\omega_{UV}\omega_S\omega_M)^2 + (\omega_{UV}\omega_S\omega_L)^2 + (\omega_{UV}\omega_M\omega_L)^2 + (\omega_S\omega_M\omega_L)^2}}$$

for hue, and

$$\text{JND} = \Delta S = \left| \frac{\Delta f_D}{\omega_D} \right|$$

for luminance (Siddiqi *et al.* 2004), where Δf represents the logarithmic ratio of photon captures from the focal pair of colours by a given type of photoreceptors (i.e. $\log \frac{Q_i}{Q_j}$), and ω represents the relative abundance of each cone type in the posterior dorsal area of the retina, incorporating the Weber fraction of 0.05 (an error rate in the Weber–Fechner law by convention) (Vorobyev *et al.* 1998). We set a JND of 2 as the standard for the minimum discriminability, which was stricter than those in most visual modelling studies, as we were testing the lack of differences between the morphotypes. The reflectance spectra were drawn with the ‘pavo’ package (Maia *et al.* 2013), and the tetrahedral plots were drawn with the ‘lattice’ package (Sarker 2008), both in the R software, version 3.1.1 (R Foundation for Statistical Computing, Vienna; <http://www.r-project.org/>).

Statistics

Representative values (logarithmic means) for photon captures were estimated using log-linear mixed-effect models with the ‘lme4’ package (<http://cran.r-project.org/package=lme4>) in R. We assigned individual plant as a random effect, but did not assign collection site because its explanatory power was too small in these models. Deterministic JNDs between morphotypes were calculated from exponential-transformed parameter estimates for photon captures in these models.

To evaluate the variance of discriminability between measured colours, we first calculated JNDs between all possible combinations of measured colours. The number of JNDs amounted to 97 020 (the cumulative sum of $n - 1$, i.e. $\frac{n(n-1)}{2}$, where $n = 441$) for within-organ comparisons (i.e. fruit–fruit or leaf–leaf), and 194 922 (n^2) for the between-organ comparison (i.e. fruit–leaf). Next, we formulated distance matrices from all the

calculated JNDs, and then converted these matrices into given sets of coordinates of a size n (i.e. 441 for fruit and leaf, respectively) through the principal coordinate analysis (PCoA; Legendre & Legendre 1998). We used the ‘cmdscale’ command default in R, setting the dimensional parameter ‘k’ as 3 since the original colour data scattered in a tridimensional space. Each measured colour thus had three unique coordinates,

which represent its relative positions on respective axes among all the analysed colours. We chose given sets of coordinates with eigenvalues greater than 1 to analyse. First principal coordinates for both hue and luminance JNDs were integrated in a principal component analysis using a default command ‘prcomp’ in R to estimate the discriminability of colours of the objects to birds as a whole, since birds might not perceive the two aspects of colour separately (Osorio *et al.* 1999).

We then compared Bayesian posterior probabilities for these first principal coordinates/components between morphotypes inferred with the ‘MCMCglmm’ package (Hadfield 2010), in which plant identity and collection site were assigned as random effects. The number of iterations was set as 510 000, the thinning interval 1000, and the burn-in (pilot run) 10 000. The total Markov-chain Monte-Carlo (MCMC) samples were thus 500 for each parameter. From the inferred posterior probabilities, we estimated the difference in the discriminability between C– and C+, and calculated the probability that the differences of posterior means were greater than 0 (i.e. proportion of cases in which the difference of each MCMC sampled C– from that of C+ was below 0). Note that a statistically significant difference is not always related to the difference in perceived colours, as colours with JNDs less than 2 would be indiscernible to the viewer (Siddiqi *et al.* 2004), irrespective of whether such a difference is biologically substantial. Distributions with negative means were negative-transformed to aid interpretation.

RESULTS

Representative photon capture and deterministic JND

Although photon captures by some single-cone types differed significantly between C+ and C– (fruit UVS: $\chi^2_1 = 6.49$, $P = 0.011$; leaf UVS: $\chi^2_1 = 25.1$,

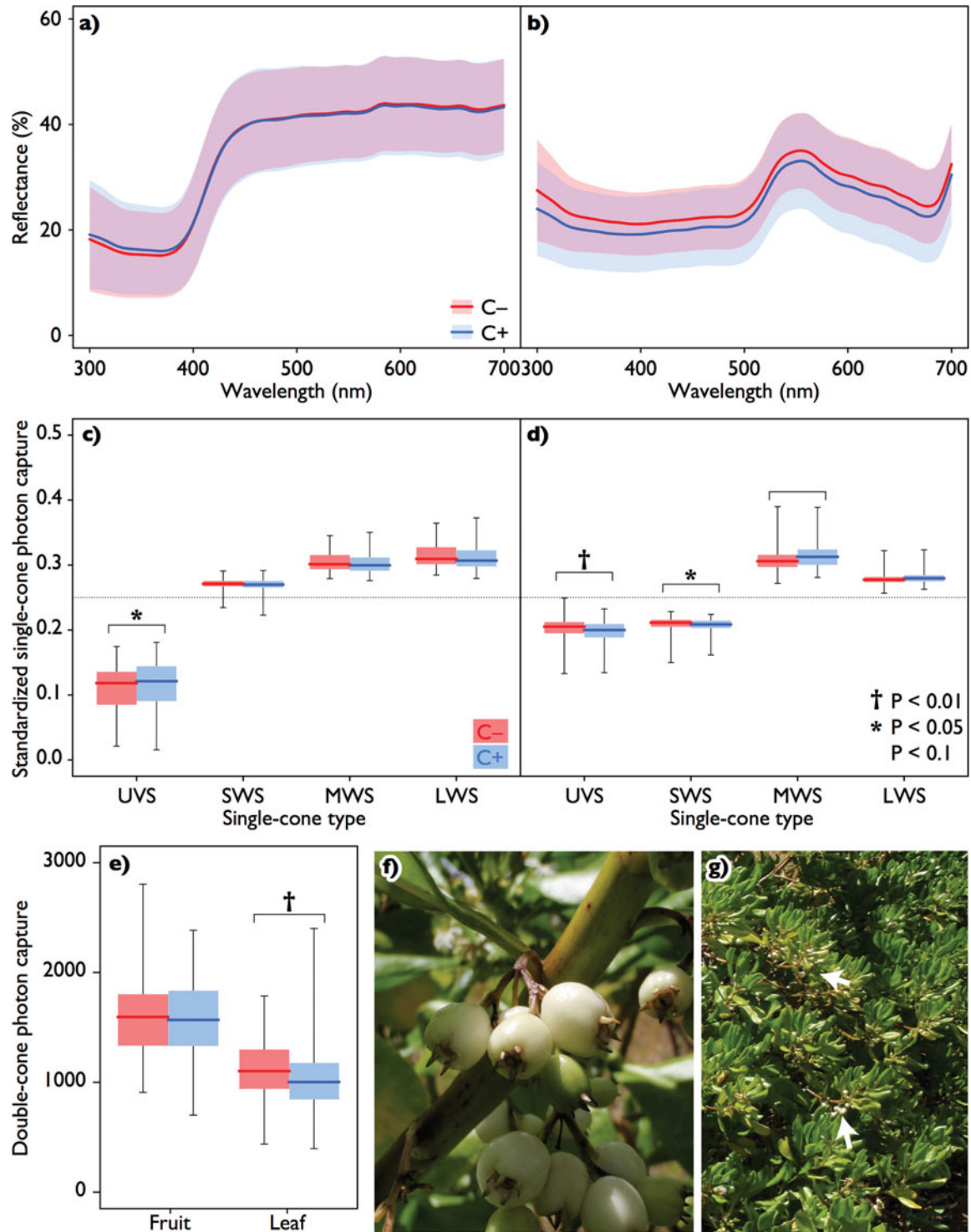


Figure 1. Mean reflectance spectra of light from surfaces of fruits (a) and leaves (b) of *Scaevola taccada* with standard deviations for respective morphotypes. Line colours denote the endocarp morphotypes: red for the one without cork (C-) and blue for that with cork (C+), and translucent areas denote ranges of each SD (purple for overlapped ranges). Photon captures by single-cone types (UVS: ultraviolet sensitive; SWS: short-wavelength sensitive; MWS: middle-wavelength sensitive; LWS: long-wavelength sensitive) from the measured reflectance from fruits (c) and leaves (d) and those by double-cones (e). Boxes are composed of medians with 1st and 3rd quartiles, with whiskers indicating ranges. Dotted lines indicate the level of standardized photon capture for avian grey. Symbols denote the significance level of differences between morphotypes. *Scaevola taccada* fruits of C+ (f), and the fruiting plant with bunches of ripe fruits indicated by arrows (g), photographed in Canala, New Caledonia.

Table 1. Between-organ deterministic JNDs (just noticeable differences) for chromatic (hue) and achromatic (luminance) aspects of the colours of fruits and leaves of *Scaevola taccada* in relation to the endocarp morphotype with (C+) and without cork (C-) found in the main island of Okinawa, in the vision of potential avian seed dispersers. The vision of seed dispersers was approximated based on photoreceptor sensitivity of the blue tit and the avian tetrachromatic colour space.

		Hue		Luminance	
		Leaf		Leaf	
		C-	C+	C-	C+
Fruit	C-	8.98	8.72	8.21	6.85
	C+	8.49	8.24	8.34	6.98

$P < 0.0001$; leaf SWS: $\chi^2_1 = 5.74$, $P = 0.016$; $P > 0.05$ for all else; **Figure 1c–d**), no JND greater than 2 was detected in the within-organ comparisons: 0.54 and 0.13 for fruit hue and luminance respectively, and 0.59 and 1.36 for leaf hue and luminance, respectively. Photon capture from leaves by double-cones was significantly different between these two morphotypes ($\chi^2_1 = 7.17$, $P = 0.007$), while those from the fruit did not ($\chi^2_1 = 0.096$, $P = 0.76$; **Figure 1e**). Again, no JND greater than 2 was detected between leaves of the two morphs. JNDs between fruits and leaves of any combinations of morphotypes were much greater than 2 both in hue and luminance (**Table 1**).

Discriminability with variance

The distribution of measured colours was different between fruits and leaves, but apparently not between the endocarp morphotypes in the avian tetrachromatic colour space (**Figure 2**). The distributions of first principal coordinates/components for JNDs appeared not to differ between the morphotypes (**Figure 3**). Eigenvalues of all the first principal coordinates/components were exclusively greater than 1, and thus their unit could be an approximation of the original parameter (i.e. hue and luminance JNDs, though its precision varied with eigenvalues) (**Figure 3**).

All the posterior probabilities for the differences in the first principal coordinates between the morphotypes were not greater than 0 at the 5% level (**Figure 4a–f**) except in the leaf hue JND ($P = 0.048$; **Figure 4b**). Nevertheless, none of the differences between the posterior means (i.e. effect sizes for the approximate JNDs for both C- and C+) was greater than 2.

Integrated discriminability: chromatic and achromatic thresholds

The posterior probabilities of differences in the first principal components between the morphotypes, which were integrated from the first principal coordinates of hue and luminance JNDs, were not greater than 0 at the 5% level (**Figure 4g–i**), and none of the differences between posterior means was greater than 2.

DISCUSSION

Fruit colour difference between morphotypes

Though the fruit colour of *S. taccada* was highly discriminable from its leaf colour in the deterministic approach, it is certain that such a measure of differences is insufficient to infer how seed-dispersing birds can distinguish between fruits of the two morphotypes, i.e. C+ and C-, because the variability of perceived differences was not considered. Actually, the deterministic JNDs of luminance between fruit and leaf colours varied roughly with the morphotype (**Table 1**), though such a difference is untestable using conventional methods. By processing data so that such dissimilarity was quantifiable, we showed that not only the perceived dissimilarity within fruits or leaves, but the one between fruits and leaves were not different between morphotypes (**Figure 4**). These results suggest that the fruit colours of the different morphotypes would be indistinguishable from the point of view of avian seed dispersers. Thus, to avoid consuming fruits with cork, birds would have to rely on other discrimination cues, such as the size-weight relationship of fruits, or spatial locations of individual plants. In fact, frugivorous birds seem not to distinguish the morphotypes as they consumed C+ fruits (Emura *et al.* 2012, Kawakami *et al.* 2009) in areas where both morphotypes were found (Emura *et al.* 2014), and both types of seed were found in faeces and pellets of birds in our collecting sites (Emura, pers. obs.). To sum, the endocarp dimorphism is cryptic sensu stricto to frugivorous birds, and thus the fruit colour of *S. taccada* is a relatively dishonest signal about its reward.

Although we do not know the gene expression mechanism of the endocarp dimorphism of *S. taccada*, theories might help disentangle the evolutionary relationship of the dimorphism and the dishonesty (Gigord *et al.* 2001, Gray & McKinnon 2007). For example, we would assume that Mendel's law of dominance would apply both for C+ and a putative mutant allele for a novel fruit colour, and both are genetically uncorrelated traits. Although the novel colour gene seems to be adaptive, this new colour morph still could not provide an honest signal to seed dispersers because fruit colours

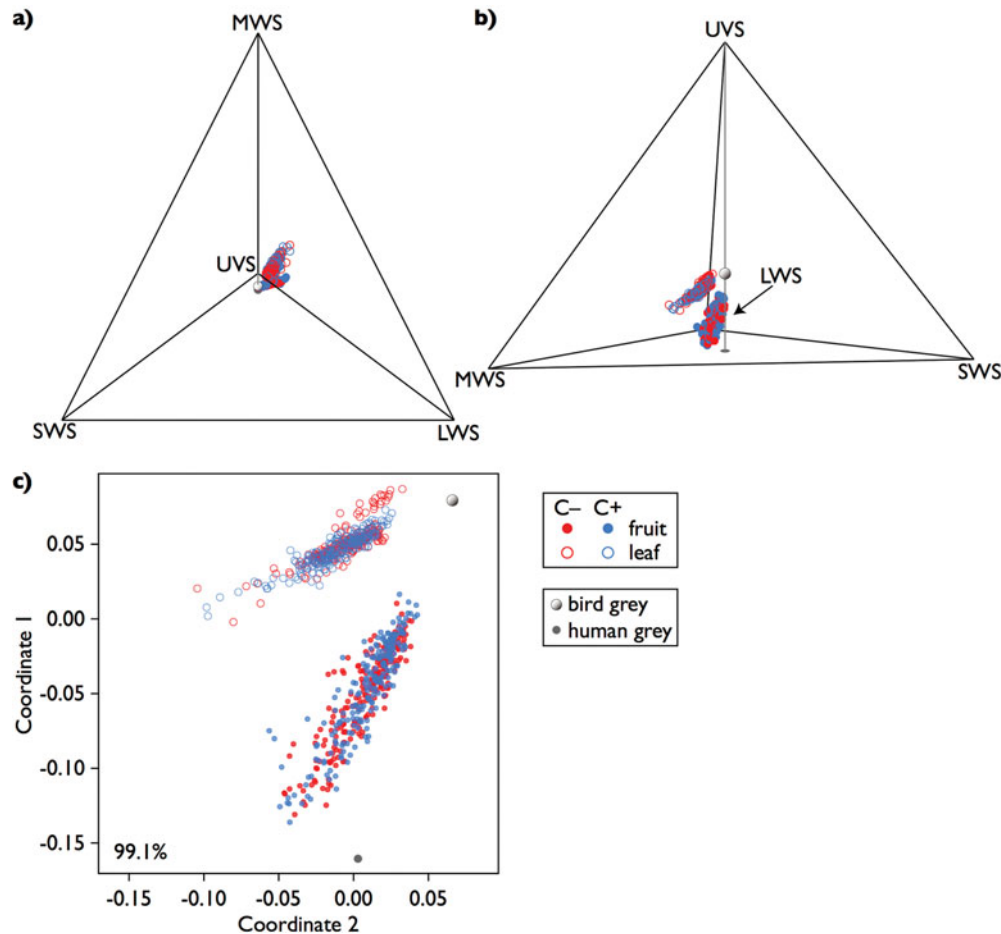


Figure 2. Colours of fruits and leaves of *Scaevola taccada* in the avian tetrachromatic colour space. Viewing angles are altered to illustrate the aspect of avian tetrachromatic hue that is visible to humans (a), and that invisible to humans (b). Dot colours denote the endocarp morphotypes, red for the one without cork (C-) and blue for that with cork (C+); filled circles denote fruits, and open circles leaves. Single-cone types (UVS: ultraviolet sensitive; SWS: short-wavelength sensitive; MWS: middle-wavelength sensitive; LWS: long-wavelength sensitive) are labelled at apex where inputs from respective photoreceptors contribute solely to perceived hue. The arrow indicates the apex for LWS. Two-dimensional coordinates representing Euclidean distances between each measured colour in the tetrachromatic colour space (c), converted with the principal coordinate analysis. The percentage denotes the proportion of variance explained jointly by the first and second principal coordinates.

are expected to segregate independently of the endocarp types according to Mendel's law. Therefore, a required condition for an honest signal is a strong genetic correlation between dominant alleles of the endocarp and fruit colour genes. However, such genetic hitchhiking would be stochastically unlikely (Barton 2000), so is the signal honesty. The low probability of genetic linkage implies that divergent selection to promote speciation is required to make the fruit colour an honest signal under endocarp dimorphism (Gray & McKinnon 2007). Therefore, any kind of habitat segregation between respective morphotypes is necessary to prevent avian seed dispersal as well as gene flows through pollination (Forsman *et al.* 2008, Gillespie *et al.* 2012, Gray & McKinnon 2007). In fact, some close relatives of *S. taccada* that have different fruit colours and lack the cork endocarp, such as *S. montana* in New Caledonia,

inhabit inland areas that are far from coastal areas where *S. taccada* is found (Howarth *et al.* 2003). Considering such genetic constraints as improbable genetic linkage or introgression, the dishonesty in the reward signal of *S. taccada* might have been formed as a byproduct, rather than for the sake of the plant. Further genetic analyses could reveal such evolutionary dynamics of *S. taccada*.

Application of the PCoA to visual modelling

The nature of colour perception by colour-sensing animals prevents application of common statistical procedures (Tanaka in press). We review statistical constraints on the visual model and discuss how to circumvent statistical problems encountered in previous studies. Few techniques exist to test the lack of difference

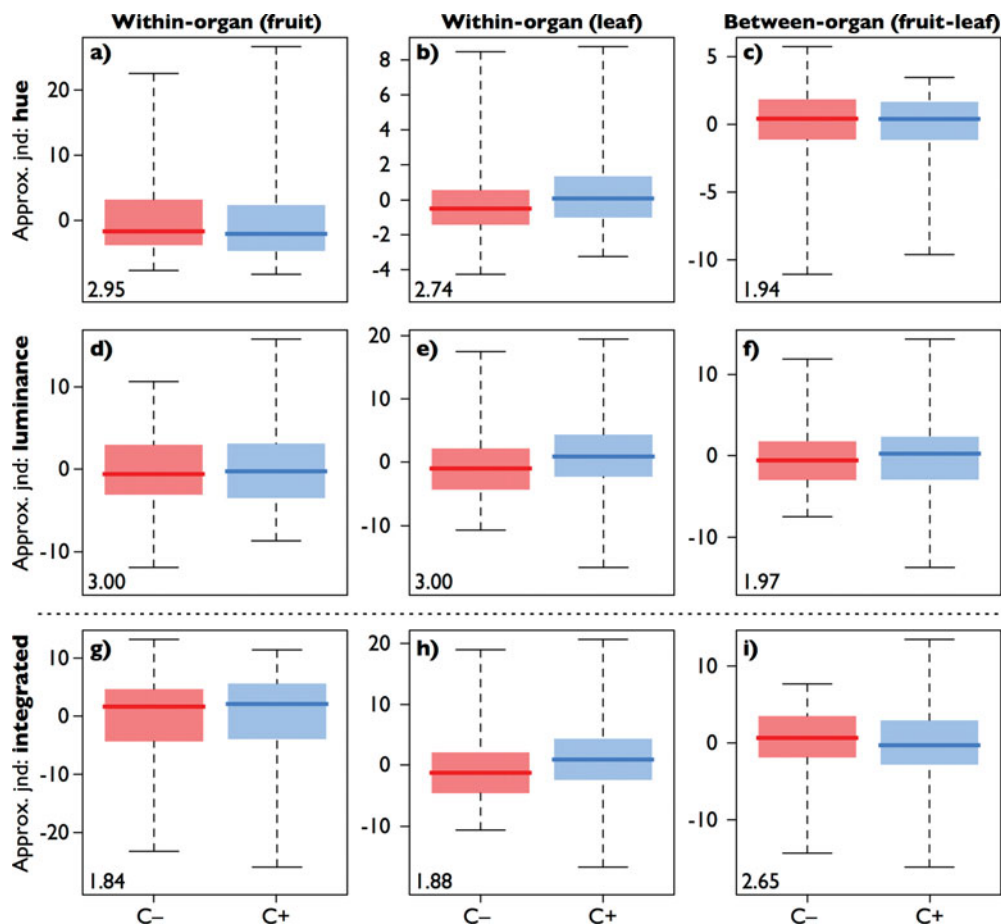


Figure 3. Distributions of first principal coordinates of hue (a–c), luminance (d–f), and integrated JNDs (first principal components; g–i) for the colours of fruits and leaves of *Scaevola taccada* in relation to the endocarp morphotype with (C+) and without cork (C–) compared within and between organs. Boxes are composed of medians with first and third quartiles with whiskers indicating ranges. Numbers represent eigenvalues for respective first principal coordinates. Colour indication corresponds to those in Figure 1 and 2.

in colour contrasts between samples. In this study, we employed principal coordinate analysis (PCoA, or multi-dimensional scaling), in which a set of coordinates in a given dimension is formulated from a distance or similarity matrix (Legendre & Legendre 1998). The PCoA is particularly compatible with visual modelling because a JND is an approximated perceived dissimilarity, i.e. the psychophysical distance between given two colours (Backhaus *et al.* 1987, Vorobyev & Osorio 1998). Four major methods have been adopted in the studies of avian visual modelling to quantify colour differences: (1) representative (averaged) reflectance/photon captures and deterministic JNDs (Avilés 2008, Feeney *et al.* 2014, Tanaka *et al.* 2011); (2) estimating volumes in the colour space occupied by given colours (Langmore *et al.* 2011, Maia *et al.* 2013, Stoddard & Prum 2008, 2011; Stoddard & Stevens 2011); (3) averaging JNDs (Antonov *et al.* 2011, Avilés 2008, Avilés *et al.* 2011, Cassey *et al.* 2009); and (4) the Mantel test (Avilés *et al.* 2012, Fadzly *et al.* 2013). However, each of these methods has drawbacks.

First, the representative approach begins with calculation of averages for reflectance spectra (Avilés 2008) or photon captures (Tanaka *et al.* 2011; this study), or centroids of focal colours in the colour space (Feeney *et al.* 2014). Then, JNDs for focal colours against a given set of average photon captures (Avilés 2008, Avilés *et al.* 2011), JNDs of all colours from the centroid of the focal colours (Feeney *et al.* 2014), or JNDs between averages are calculated (Tanaka *et al.* 2011; Table 1 of the present study). Although such results are intuitive, the representative approach partially or even wholly discounts the variance of data (i.e. JNDs are deterministic), which could cause type I errors. Second, the volume estimation calculates a volume that encompasses outlying data points (i.e. minimum convex polygon) of measured colours in the tetrahedron (Maia *et al.* 2013, Stoddard & Prum 2008). While the volume estimation efficiently reduces the dimension of data, it disregards the density of distribution, which could cause type II errors by over-weighting outliers. The third

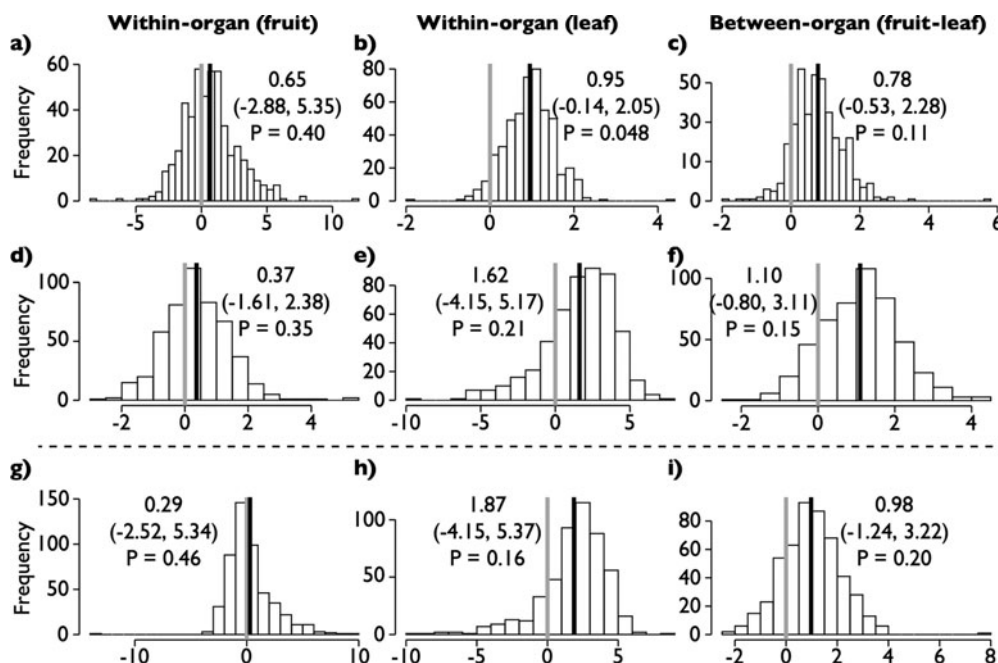


Figure 4. MCMC-sampled Bayesian posterior probabilities for the differences in first principal coordinates of hue (a–c), luminance (d–f), and integrated JNDs (first principal components; g–i) for the colours of fruits and leaves of *Scaevola taccada* in relation to the endocarp morphotypes with (C+) and without cork (C–) compared within and between organs. Values represent the effect sizes (posterior means) with lower and upper ends of the 95% credible intervals in the parentheses, with probabilities that the posterior means are statistically different from 0. Solid lines indicate the mean, and grey lines 0. Distributions with negative means were negative-transformed to aid interpretation. The positions of panels correspond to those in Figure 3.

method, averaging JNDs, treats each JND value as a repeated measurement, and then analyses the effects of covariates on the set of the JND values (i.e. as a response variable) with a linear model (Cassey *et al.* 2009). However, the averaging of JNDs severely violates two fundamental principles of statistics: (1) the congruence principle, and (2) the independence of data. In reference to the congruence principle, treating a set of (Euclidean) distances as a linear variable is to ignore the mutual geometric dependence of each datum, which is non-existent in linear variables (Biondini *et al.* 1991, Endler & Mielke 2005, Endler *et al.* 2005). With regard to the independence principle, each colour has $n - 1$ JNDs, so the number of analysed JNDs in this procedure must be $n(n - 1)$. However, a single JND always stems from a pair of measured colours, so the total number of JNDs cannot be the sum of the number of JNDs that are related to each measured colour. The original number of JNDs is the cumulative sum of $n - 1$, i.e. $\frac{n(n-1)}{2}$, and thus the number of analysed JNDs will be literally duplicated.

Finally, the Mantel test should be statistically more robust than the other methods as it satisfies the congruence principle, and thus is capable of directly analysing distance matrices. However, the Mantel test is designed to estimate correlation between two distance matrices, and thus causal relationships could not easily be detected. Moreover, the Mantel test is not applicable to

longitudinal data (i.e. repeated measurements), and thus pseudoreplication is unavoidable. Repeated measurement is the strongest way to reduce measurement errors (Bolker *et al.* 2009), and is especially important in the visual modelling framework since such errors could occur at a scale beyond human perception. The partial Mantel test might do better at avoiding pseudoreplication, although its validity is controversial (Raufaste & Rousset 2001).

The PCoA, or MDS, clearly has superiority over the procedures described above, because of its statistical validity. The concept of the PCoA is similar to that of the Mantel test, in that both can analyse distance matrices, but different in that the PCoA yields linear coordinates from distance matrices (Legendre & Legendre 1998). The resultant set of coordinates corresponds exactly to a well-known normally distributed variable, the principal component, and is thus compatible with linear models, currently the commonest framework in biostatistics (Bolker *et al.* 2009). Even though the PCoA does have some drawbacks, such as the reduction of dimensions inevitably entailing missing information, it could reduce the risk of false discoveries or false rejections of null hypotheses, while avoiding statistical violations such as incongruence or pseudoreplication. To sum, applying the PCoA to visual modelling analyses could expand the application of hypothesis testing statistically more robustly, particularly when the data structure is

so highly multi-dimensional as in avian tetrachromatic colours (Backhaus *et al.* 1987).

Conclusion

Our study revealed that the fruit colours of *S. taccada* are not discernibly different to avian seed dispersers between morphotypes, based on the outputs from our robust statistical modelling. Thus, the seed-dispersal strategy of C+ individuals of the plant is likely to result in a dishonest signal, which can impose greater costs on the avian seed dispersers. However, it is not certain that the signalling dishonesty exists solely for the benefit of the plant, rather than as a byproduct of other factors.

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