# Evaluating Frugivore-fruit Interactions Using Avian Eye Modelling

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**Abstrak:** Fenotip buah biasanya dihipotesiskan dengan pemilihan oleh frugivor. Kami menguji dua hipotesis mengenai interaksi frugivor-buah dari perspektif warna buah. Kami mengambil bacaan spektra 26 buah dan daunnya dari 2 pulau di New Zealand. Pertama, kami menguji hipotesis buah-dedaun; warna buah dianggap bahawa terhalang secara evolusi oleh daun buah itu sendiri untuk memaksimakan kontra warna dan kejelasan buah. Kami menjalankan analisa model sifar perbandingan kontra warna buah berdasarkan model mata burung. Kedua, kami menguji hipotesis kekhususan frugivor; warna buah yang khusus dianggap berkaitan dengan burung frugivor yang khusus. Kami menjalankan regressi bilangan kunjungan burung dengan warna buah di dalam ruang warna tetrahedral berdasarkan pengiraan mata burung menggunakan ujian Mantel. Keputusan menunjukkan bahawa warna buah adalah tidak terhalang oleh warna daunnya sendiri. Tidak ada sebarang hubungan atau corak yang mencadangkan kaitan antara buah khusus dengan burung yang berkunjung khusus. Kami mencadangkan walaupun warna buah adalah satu komponen yang sering dibincangkan, warna buah bukan merupakan satu-satunya faktor penentu di dalam interaksi frugivor-buah.

**Kata kunci:** Warna Buah, Warna Daun, Interaksi Frugivor, Model Mata Burung

**Abstract:** Fruit phenotypes are often hypothesised to be affected by selection by frugivores. Here, we tested two hypotheses concerning frugivore-fruit interactions from the perspective of fruit colours. We measured the spectral properties of 26 fruits and the associated leaves of plants from 2 islands in New Zealand. Visual observations were also performed to record the birds that fed on the fruits. First, we tested the fruit-foliage hypothesis, where fruit colours are assumed to be evolutionarily constrained by their own leaf colour to maximise colour contrast and fruit conspicuousness. We ran a null model analysis comparing fruit colour contrast using an avian eye model. Second, we tested the frugivore specificity hypothesis, where specific fruit colours are thought to be connected with a specific bird frugivore. We performed a regression on the number of bird visits against the fruit colour in tetrahedral colour space based on an avian eye calculation using Mantel's test. The results show that fruit colours are not constrained by their own leaf colours. There is also no relationship or pattern suggesting a link between a specific fruit colour and specific bird visitors. We suggest that although fruit colour is one of the most highly discussed components, it is not the most important single deciding factor in frugivore fruit selection.

**Keywords:** Fruit Colour, Leaf Colour, Frugivore Interaction, Avian Eye Model

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## INTRODUCTION

Plants produce fleshy fruits that, when consumed by animals, act as seed vectors. The sizes, shapes and colours of fruits vary enormously among plant species, and a diverse array of animals (including fish, mammals, reptiles, insects and birds) include fleshy fruits in their diets. Fruit phenotypes have long been hypothesised to be the result of selection by different types of frugivores. However, support for this hypothesis is equivocal.

Plants are often brightly coloured to attract frugivores and pollinators (see Pohl *et al.* 2008; Raine & Chittka 2007; Burns & Dalen 2002; Kelber 1996; Willson *et al.* 1990; Janson 1983; van der Pijl 1972). While colour was initially an abstract component, the advancement of animal eye photoreceptor sensitivities has enabled colour to be quantitatively assessed based on the animal's eye receptor capabilities (Endler & Mielke 2005; Hart 2001; Hart *et al.* 2000; Vorobyev *et al.* 1998; Chittka 1992; Backhaus 1991).

Here, we tested two hypotheses predicting that the colours of fleshy fruits result from selection by seed-dispersing birds based on the capabilities of avian eye receptors. First, we tested the fruit-foliage contrast hypothesis, which predicts that fruit colours are more conspicuous when displayed against their own leaves relative to leaves of other species. For example, Burns and Dalen (2002) found that the colours of fruits in coastal British Columbia, Canada, vary over time according to the seasonal changes in the colour of the foliage. Similarly, Lee *et al.* (1994) found that the colour of New Zealand Coprosma varies interspecifically with leaf size. Larger-leaved species at lower altitudes tend to have red fruits, perhaps to maximise their conspicuousness against green backgrounds. However, Schaefer *et al.* (2007) rejected the fruit-foliage hypothesis when fruit conspicuousness was tested using an avian eye model. More recently, Burns *et al.* (2009) found little support for the fruit-foliage contrast hypothesis in five geographic locales scattered across the globe using a similar avian eye model method.

Fruit colouration is the most studied plant signal (Lomascolo & Schaefer 2010; Lomascolo *et al.* 2008; Schaefer *et al.* 2004; Voigt *et al.* 2004). While some studies have shown that frugivores display consistent colour preferences (Lomascolo & Schaefer 2010; Lomascolo *et al.* 2008; Burns 2005; Burns & Dalen 2002), others have documented variable fruit colour preferences (Schmidt *et al.* 2004; Dominy & Lucas 2001). However, the question of why fleshy fruits come in a wide assortment of colours is still open for discussion (Whitney 2005; Whitney & Lister 2004). Selection on fruit colour could drive diversification in fruit colours. For example, if a plant species had a wide distribution, overlapping two distinct ranges of dispersers, and variance in genes for the colour of fruit produced, then different colour preferences of the disperser animals could promote fruit colour diversification. Strong and opposing selective pressures from the dispersers would favour different fruit colours across the species' ranges. Eventually, two emergent species would form, with a low-fitness hybrid zone (where mating between the two species would produce a fruit colour not favoured by either disperser) that reinforces the diversification. Selective pressures such as these could produce a diversification in fruit colours. The second hypothesis we tested

to explain fruit colour diversity is referred to as the "frugivore specificity hypothesis," where different frugivorous bird species are hypothesised to select different colours. For example, a specific avian species might be attracted to red fruits, while another species is attracted to white fruits. The availability of information on animal eye photoreceptor sensitivities provides a new method to evaluate this hypothesis. We performed a regression analysis between colour (based on bird eye cone excitation values) and the number of bird visits.

We measured the spectral properties of fruits and leaves from 26 species of plants from 2 main islands (North and South) in New Zealand. Observations were also made on the number of bird species that feed on the fruits. We conducted spectrometric analyses based on the avian eye model to assess whether 1) leaves constrain the colour of fleshy fruits based on the fruit-foliage hypothesis and 2) specific fruit colours are preferred by specific birds.

## MATERIALS AND METHODS

### Fruit Colour Analyses
A total of 26 species of fleshy-fruited plants were sampled from 2 study sites in New Zealand. Nine species were sampled from Nelson Lake National Park, South Island (41°81'S, 172°85'E), and 17 species were sampled from Otari-Wilton's Bush, Wellington, North Island (41º15'S, 174º45'E). Nelson Lake receives approximately 1000 mm of rainfall per year, and frosts and snow are common in the winter. The vegetation consists predominantly of Nothofagus trees. There is high diversity of small trees and shrubs, mostly dispersed by birds. Most of the forested area remains undisturbed by human activities. Otari-Wilton's bush receives annual rainfall of 1250 mm. The forested area consists of mature and regenerating conifer broadleaf forest (see Burns & Dawson 2005). The sampled plants were the most common fleshy-fruited species at either site and were included on because they were encountered during foraging observations of fruit-eating birds. Five fruit and leaf samples were taken from each of five individual plants from all species. For each fruit and leaf collected, five spectrometric readings were taken and averaged prior to analysis.

The spectral analysis of each fruit and leaf was conducted using a USB OceanOptics 2000 (OceanOptics, Florida, USA) spectroradiometer, and a Xenon Pulse X2 (OceanOptics, Florida, USA) lamp was used as the light source. The reflection was measured as the proportion of a Diffuse Reflectance Standard (white standard). The fibre optics probe was mounted inside a matte black plastic tube to exclude biases caused by ambient light. The distance between the probe and the leaf or fruit was set at I cm. The angle of illumination and reflection was fixed at 45° to minimise the objects' glare. The spectra were processed with SpectraSuite (OceanOptics, Florida, USA) software and calculated in 5 nm intervals from 300 to 700 nm. The irradiance was measured by using a cosine corrected sensor, and a D65 light (normal daylight) bulb was used as a reference.

We used an eye model based on the spectral sensitivities and receptor noise of the four cone types possessed by birds (*u, s, m* and *l*) to quantify the

fruit and leaf colours as they would appear to a typical avian frugivore. We quantified the appearance of leaves and fruits using the contrast comparison method, which follows simple colour pattern measures related to photon capture (Endler & Mielke 2005; Vorobyev *et al.* 1998). Detailed explanations of the mathematical formulae are given elsewhere (Vorobyev & Osorio 1998; Osorio & Vorobyev 1996), but the model is sufficient to predict the discriminability of any two of spectra, provided only that the receptor spectral sensitivities and noise can be estimated (refer to Appendix 1 for further calculations). The calculation provides photon capture values for each type of cone receptors in the bird's eye. The receptor spectral sensitivity values were obtained from Endler and Mielke (2005).

Colour is defined as a point in a perceptual space whose co-ordinate axes represent quantum catches of receptors (Poirson & Wandell 1990). An object's appearance is a function of two components, the chromatic (wavelengths) and achromatic (brightness) contrasts between an object and its spectral background. The colours we can see are chromatic; for example, the colour red is within the 600–700 nm wavelength. Achromatism, or brightness, refers to the intensity of the colour itself (for example; dark red or light red). We used the tetrahedral transformation method (refer to Endler & Mielke 2005; Aitchison 2003) to characterise the chromatic contrasts. First, the output of each of the four cones (*u, s, m* and *l*) was transformed into points in tetrahedral space with a height of 1, resulting in *x, y* and *z* Cartesian coordinates in three-dimensional space.

$$x = \frac{1-2s-m-u}{2}\sqrt{\frac{3}{2}} \ , \quad y = \frac{-1+3m+u}{2\sqrt{2}} \ , \quad z = u - \frac{1}{4}$$

The Euclidean distance between any two points in tetrahedral colour space (C) represents the difference between the chromatic components of their appearances.

$$C = \sqrt{(x_a - x_b)^2 + (y_a - y_b)^2 + (z_a - z_b)^2}$$

For example, a large Euclidean distance between the points representing a fruit and its corresponding leaf background would indicate a chromatically conspicuous fruit display. Achromatic (brightness) contrasts were calculated differently because the exact working nature of achromatic signals in birds is still poorly understood (Hart 2001; Osorio *et al.* 1999; Campenhausen & Kirschfeld 1998). Some birds have double cones with broad spectral sensitivities that overlap with both long and medium wavelength-sensitive cones, and these double cones facilitate achromatic signal processing (non-colour based tasks) (Cuthill 2006; Jones & Osorio 2004; Hart *et al.* 2000). Achromatic contrasts (ΔS) were calculated as:

$$\Delta S = \left| \Delta f_i \, / \, \omega_D \right|$$

$\Delta f_i$ represents the difference between two objects in their capacity to stimulate receptor mechanisms (refer to Vorobyev *et al.* 1998; Osorio & Vorobyev 1996) and $\omega_D$ represents the value of double cones. Because $\omega_D$ is the same for all targets, it does not affect relative achromatic contrasts. The only available double cone receptor data sensitivity is based on *Leiothrix lutea,* and the $\omega_D$ is therefore valued at 0.05. The discriminability of any two colours is described by the "distance," $\Delta f_i$ between them in "just noticeable differences" (JND) units. This method sometimes produces negative values, which simply indicates that one object is darker than the other, and its sign depends on which reflectance data set is entered into the calculation first. Absolute values were used for the statistical analysis. All of the eye model calculations and statistical analyses were conducted in R 2.10.1 (R Development Core Team 2010).

**Frugivore Sampling**
Observations of birds foraging for fruit were conducted using the protocol described by Burns and Lake (2009) and Burns (2006a). Over the course of three fruiting seasons on the North Island (November to June from 2006–2008) and two fruiting seasons on the South Island (March to May from 2007–2008), we visited a series of trails and observed birds foraging for fruit. We classified an observation as a "visit" when a bird approached a plant and consumed at least one fruit. Observations were halted after each sighting to avoid repetition, and observations continued again 10 m further along the path at different trees. Each observation session was conducted from 8.00 a.m. until 11.00 a.m. There were three trails selected at Nelson Lakes (two hiking trails, one leading up to St Arnaud Mountain and the other to Mount Roberts, and one walking trail along Lake Rotoiti). Four trails were used at the Otari-Wilton's Bush area (one walking trail around the native garden area and three sections of the hiking trail inside the bush area). More than 80 hours of observation were conducted in the South Island, and more than 100 hours were conducted on the North Island. The fruiting season on the North Island (November-June) is more protracted than that of the South Island (March-May) (Burns & Lake 2009), so a longer observation period was required on the North Island to adequately characterise bird-fruit interactions.

**Statistical Analysis**
First, we examined fruit colour variation by comparing the chromatic and achromatic values of the fruits gathered from North and South Island. The values were compared statistically using a t-test. Second, to test the fruit-foliage hypothesis, we compared the chromatic and achromatic contrast of a fruit matched with its own leaf (observed value) against the average contrast value from the same fruit matched with the leaves of other plants excluding its own (expected value). In contrast to the methods used by Burns *et al.* (2009), we suggest that the null model value should be from the comparison of a specific fruit (for example, fruit *a*) against the leaf reflectance of all other fruits (excluding

fruit *a*'s leaf reflectance). The values were compared statistically using ANOVA with the observed and expected values designated as the dependent variables. To test the geographical factors, we set the locality (North or South) as the random factor. Because the single observed value is compared against the expected values, which are derived from an average (calculated from (n–1) number of plants), there might be variability in the expected value data spread. To resolve this issue, we calculated z score values from each of the expected results. We then performed a sample t-test on the z score values with the test value set at 0. An  insignificant result would show that data variability has no effect on the overall results. The test was performed on both chromatic and achromatic comparisons for North and South Islands.

Third, to test the frugivore specificity hypothesis, we composed two matrices (for North and South Islands) based on the number of visitations by a bird species to a specific plant species. The values were first transformed (using square root transformation) to conform to data normality and homogeneity. Because certain birds only visit certain plants for each island group, our data matrix is prone to heteroscedasticity. We experimented with several data transformation methods, but these methods did not qualitatively change the results, so we only report here the results of the analysis based on the square root transformation method.

We then conducted Proximity Scaling (PROXSCAL) multidimensional scaling (MDS) following the methods of Beaumont and Burns (2009). The MDS analysis was conducted separately for the North and South Islands and the corresponding information was then transformed into two dimensions. Each data point represents a plant from both islands that was plotted in a two-dimensional area. Points situated close together represent fruits that might share the same bird visitors, while widely separated points represent a different type of bird visitors. We then proceeded to calculate the Euclidean distances between each of the points. We converted the fruit reflectance values into coordinates in tetrahedral colour space, so that each fruit colour was represented by *x, y* and *z* coordinates in tetrahedral colour space. Points that are situated close together represent fruits that are identical in colour, while widely separated points represent differently coloured fruits. Similar to the previous method, we calculated the Euclidean distances between each of the points. Both groups of values (the distance values obtained from the MDS and the distance values obtained from the tetrahedral colour space analysis) were first transformed into matrices before Mantel's test was performed. Mantel's (1967) test is an approach that overcomes some of the problems inherent in explaining species-environment relationships. The calculation utilises regressions in which the variables are themselves distance or dissimilarity matrices summarising pair-wise similarities among sample locations. Mantel's test was conducted using R 2.10.1 (R Development Core Team 2010) (with the additional "ecodist" package), and each permutation was repeated 1000 times. Mantel's test was conducted separately for the North and South Islands.

## RESULTS

On the North Island, 12 species of birds (total) were recorded in 1253 observations. The relative frequencies of the 12 species of birds recorded on the North Island were as follows: waxeye *Zosterops lateralis*, n = 592; European blackbird *Turdus merula*, n = 235; tui *Prosthemadera novaeseelandiae*, n = 181; stitchbird *Notiomystis cincta*, n = 92; whitehead *Mohua albicilla*, n = 44; bellbird *Anthornis melanura*, n = 22; kaka *Nestor meridionalis*, n = 16; saddleback *Philesturnus carunculatus*, n = 62; European starling *Sturnus vulgaris*, n = 3; European songthrush *Turdus philomelos*, n = 2; malard *Anas platyrhynchos,* n = 1; and kereru *Hemiphaga novaeseelandiae*, n = 3. A total number of 158 observations were recorded for the South Island, and 6 species were observed. The relative frequency of the 6 species of birds recorded in South Island were as follows: *Z. lateralis*, n = 126; *T. merula*, n = 18; brown creeper *Mohuoua novaeseelandiae*, n = 4; *T. philomelos*, n = 3; *A. melanura*, n = 5; and *P. novaeseelandiae*, n = 2. A total of 17 species of plants from 14 families were sampled on the North Island, and 9 species from 7 families were sampled on the South Island (refer to Table 1).

There was no significant difference in the mean colour (chromatic) of North island (mean = 0.56±0.20 JND) and South island fruits (mean = 0.47±0.47 JND) (t-test = 1.174, df = 24, $p$ = 0.25). The results for achromatic contrasts between North Island (mean = 23.28±12.67 JND) and South Island fruits (mean = 25.22±21.08 JND) were also similar (t-test = –0.30, df = 24, $p$ = 0.77).

Our results do not support the fruit foliage contrast hypothesis (Fig. 1). There was no significant difference between the observed value and the expected value for chromatic contrast (ANOVA F = 7.412, df = 1, $p$ = 0.224), and this result applies to both the North and South Islands based on the locality interaction (ANOVA F = 0.020, df = 1, $p$ = 0.887). Further z score distribution analysis showed no data variability effect for the North (t = 0.259, df = 16, $p$ = 0.799) and South Islands (t = –0.545, df = 8, $p$ = 0.601). Achromatic comparisons also yielded the same conclusion. There was no significant difference between the observed value and the expected value for achromatic contrast (ANOVA F = 0.692, df = 1, $p$ = 0.558). There was also no significant difference in the locality interaction (ANOVA F = 3.674, df = 1, $p$ = 0.070). A z score distribution analysis also showed no effect of data variability for the North (t = 1.533, df = 16, $p$ = 0.145) and South Islands (t = –1.789, df = 8, $p$ = 0.111).

The MDS analysis showed that the plants are distributed across the two dimensions with some degree of clumping in some species and scattered data points based on geographical location (Fig. 2). Because MDS reduces the information into two dimensions, this inevitably results in the loss of some information. An inverse goodness-of-fit stress measure is needed to determine the accuracy of the two dimensions. Based on the Normalised Raw Stress value of 0.001 for both the North and South Islands' MDS, the plot dimensions appear accurate. Sturrock and Rocha (2000) reported that a stress value of 0.1 or lower is a good indication of plot dimension accuracy. Figure 3 shows the fruit colour distribution in a tetrahedral colour space. Mantel's test correlates the distance values within these two graphs (Figs. 2 and 3). Simulated correlation results for

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North Island (Mantelr = 0.025, *p* = 0.144) and South Island (Mantelr = 0.101, *p* = 0.963) show that the null hypothesis could not be rejected, and the MDS distances and the tetrahedral distances are unrelated at alpha = 0.05 (Fig. 4). This suggests that there is no significant support for the frugivore specificity hypothesis.

**Table 1:** Full list of plants (with abbreviations used for other figures), sampled location, and the chromatic and achromatic contrasts (observed values) along with the total number of bird visits (values in brackets refer to standard error values).

| Name | Abbreviation | Plant location | Chromatic contrast (SE = ±0.19) | Achromatic contrast (SE = ±15.71) | Total number of bird visits |
|---|---|---|---|---|---|
| | | | Euclidean distance | JND | |
| *Coprosma grandifolia* | C.g | North Island | 0.61 | 12.5 | 118 |
| *Melicytus ramiflorus* | M.r | North Island | 0.6 | –27.98 | 347 |
| *Solanum aviculare* | S.a | North Island | 0.31 | 14.55 | 5 |
| *Coprosma robusta* | C.r | North Island | 0.75 | 27.34 | 218 |
| *Pratia angulata* | P.an | North Island | 0.61 | –33.41 | 1 |
| *Aristottelia serrata* | A.s | North Island | 0.41 | –23.53 | 13 |
| *Coprosma repens* | C.re | North Island | 0.77 | 41.47 | 2 |
| *Podocarpus acutifolius* | P.ac | North Island | 0.71 | –20.32 | 25 |
| *Passiflora tetrandra* | P.t | North Island | 0.99 | 49.09 | 3 |
| *Coriaria arborea* | C.a | North Island | 0.35 | 6.09 | 27 |
| *Muehlenbeckia australis* | M.a | North Island | 0.32 | 22.43 | 43 |
| *Schlefera digitata* | S.d | North Island | 0.35 | –14.77 | 162 |

**Table 1:** (*continued*)

| Name | Abbreviation | Plant location | Chromatic contrast (SE = ±0.19) | Achromatic contrast (SE = ±15.71) | Total number of bird visits |
|------|-------------|----------------|--------------------|--------------------|--------|
| | | | Euclidean distance | JND | |
| *Pseudopanax arboreus* | P.a | North Island | 0.46 | −13.71 | 137 |
| *Hedycrya arboreus* | H.a | North Island | 0.71 | 30.78 | 16 |
| *Myoporum laetum* | M.l | North Island | 0.46 | 12.96 | 55 |
| *Ripogonum scandens* | R.s | North Island | 0.75 | −5.06 | 4 |
| *Pittosporum euginoides* | P.e | North Island | 0.35 | −39.77 | 77 |
| *Griselinia littoralis* | G.l | South Island | 0.44 | −45.99 | 5 |
| *Coprosma linariifolia* | C.l | South Island | 0.38 | 32.92 | 6 |
| *Carpodetus serratus* | C.s | South Island | 0.52 | −16.94 | 17 |
| *Coprosma foetidissima* | C.f | South Island | 0.56 | 2.09 | 13 |
| *Leucopogon fraseri* | L.f | South Island | 0.29 | −0.34 | 11 |
| *Pseudopanax crassifolius* | P.c | South Island | 0.35 | −0.93 | 81 |
| *Elaocarrpus hookerianus* | E.h | South Island | 0.55 | −32.01 | 5 |
| *Halocarpus bidwillii* | H.b | South Island | 0.38 | 57.35 | 4 |
| *Pseudopanax colensoi* | P.co | South Island | 0.76 | −38.47 | 16 |
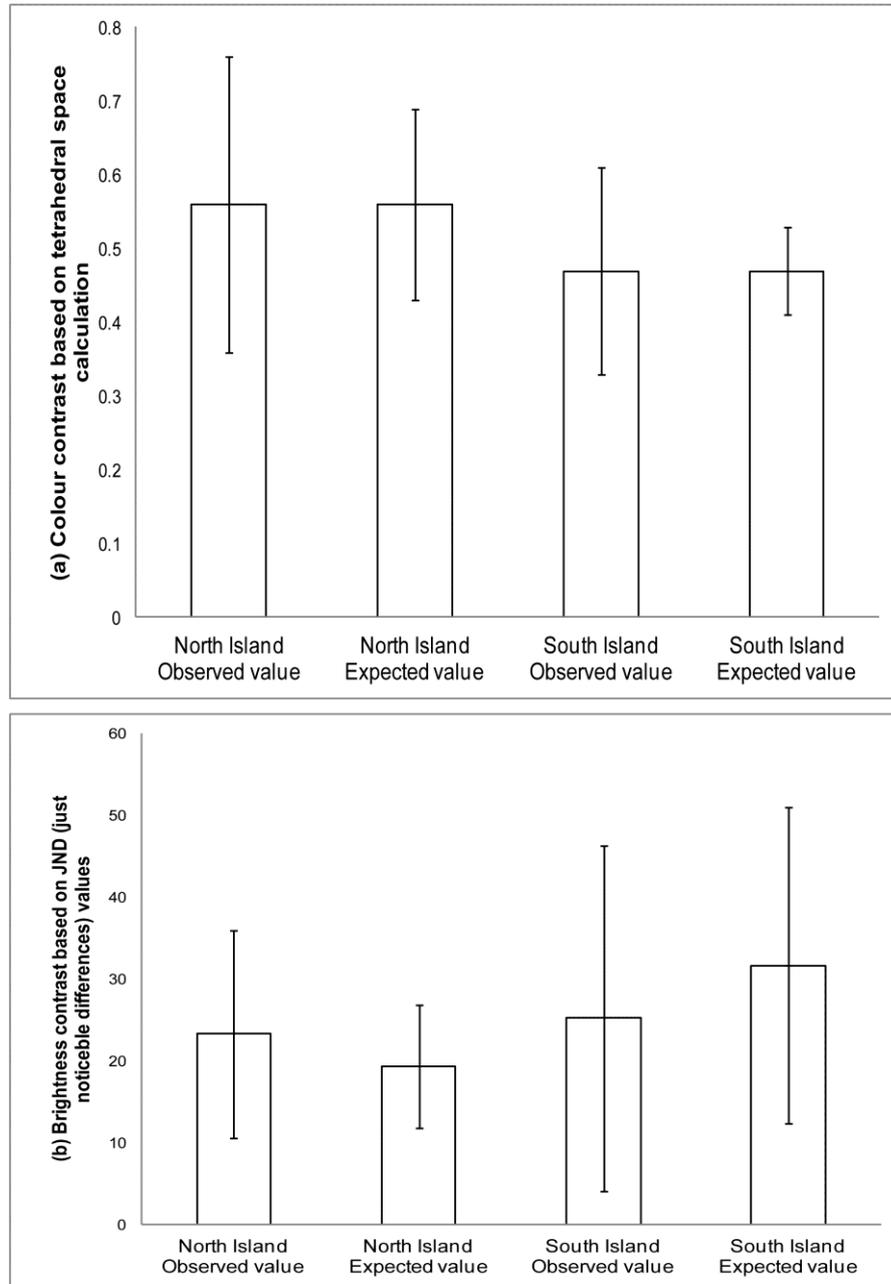
**Figure 1:** The observed and expected values of fruit colour contrast: (a) chromatic contrast refers to the Euclidean distance in tetrahedral colour space; (b) achromatic contrast refers to the JND values from the avian eye model calculation.
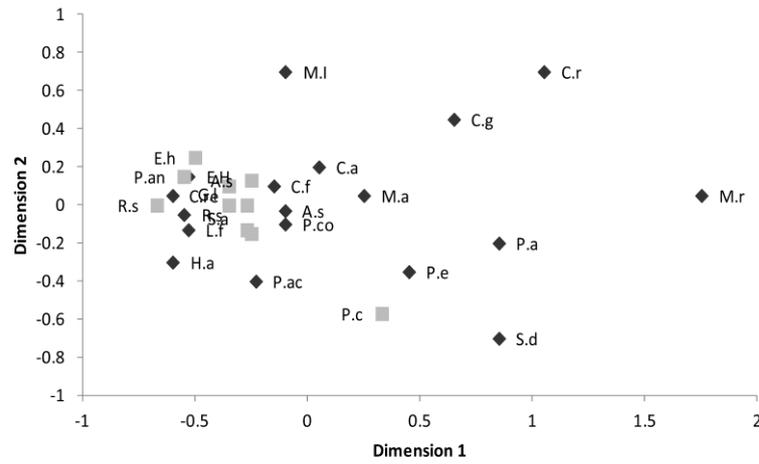
**Figure 2:** Multidimensional scaling of plant species based on bird visitation (refer to Table 1 for abbreviation descriptions; grey squares represent South Island plants and black diamonds represent North Island plants, most of the points overlap).
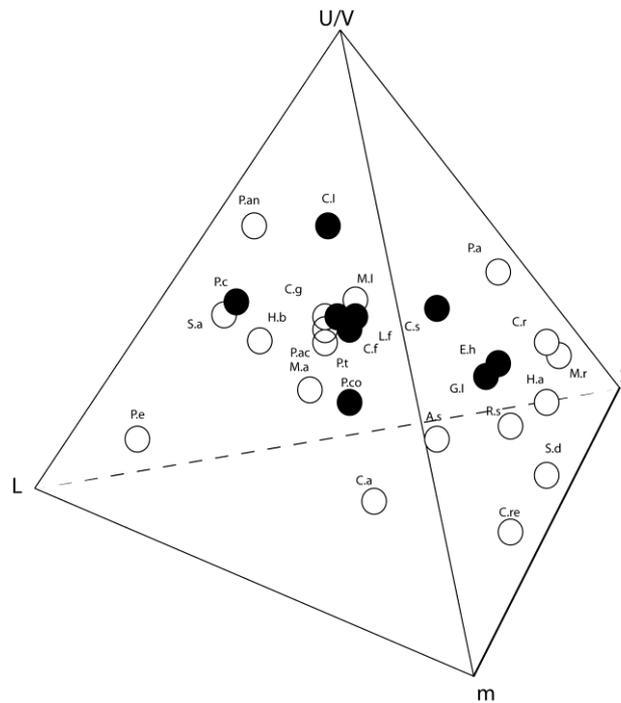


**Figure 3:** A tetrahedral colour space representation of the fruit colours. Each point represents how the specific colour of a fruit is processed by the bird eye receptors (refer to Table 1 for abbreviation descriptions; black circles represent South Island plants and open circles represent North Island plants).
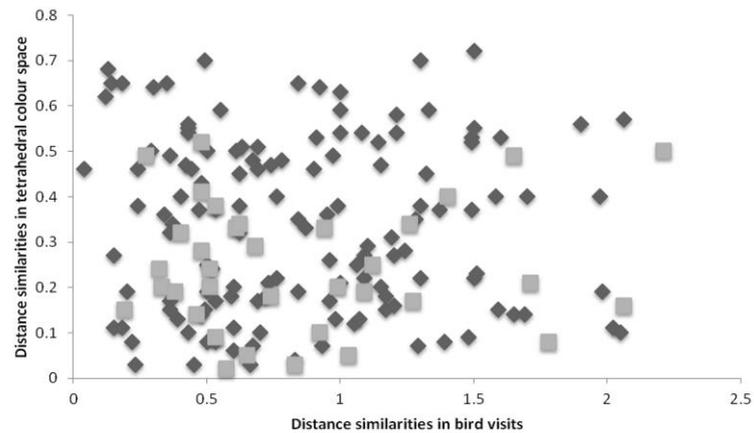
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**Figure 4:** Mantel's test correlation results for North Island (black diamonds) and South Island (grey squares) between the distances from the multidimensional scaling based on bird visits and distances in the tetrahedral colour space.


## DISCUSSION

North and South Island fruit colours were perceived similarly by birds, both chromatically and achromatically. Although the sample size is smaller than that used by Burns *et al.* (2009), both results indicate that colour foliage contrasts do not differ based on geographical location. Our results also do not support either the fruit-foliage or frugivore specificity hypothesis. The latter tested at a very fine level of specificity.

The results suggest no evidence for the fruit-foliage hypothesis, consistent with Burns *et al.* (2009). Although the Burns *et al.* (2009) hypothesis was generated based on the avian eye model, we detected a slight inaccuracy in their null model calculations. Burns *et al.* (2009) selected random points inside the tetrahedral space for comparisons between fruit colour and leaf colour and calculated the distance between them. This procedure was then iterated 1000 times using the Mathematica software (Wolfram Research, Illinois, USA). This method only generates averages of distances between points inside the tetrahedral space; it does not generate an accurate null model value. Our null model provides a more precise calculation and possible data variability issues were addressed accordingly. Furthermore, there are two components involved in colour vision, which are chromatic/colours and achromatic/brightness; the latter component was not included in the analysis performed by Burns *et al.* (2009).

A possible explanation for the rejection of the fruit-foliage hypothesis is that both leaf and fruit colours do not remain constant throughout a plants' life. Fruit and leaf colours change throughout different seasons (Archetti 2009b; Lev-Yadun & Gould 2007; Sanger 1971) due to environmental stresses (Archetti 2009a) and changes in chemical content (Schaefer & Schmidt 2004). Therefore, it is impossible for a fruit colour to remain exclusively significant only to its own leaf colour. Another possibility is that the conspicuousness of fruit colours is not

targeted exclusively for avian vision (Schaefer *et al.* 2007). Birds are known to select fruits based on content availability rather than conspicuousness (Schaefer *et al.* 2003a, b).

Our results shows that specific birds are not attracted to specific fruit colours. Burns and Lake (2009) suggest that introduced European birds might exhibit little selection for fruit colours because of their limited evolutionary history with New Zealand plants. Another possibility could be that focusing solely on fruit colours is too simplistic. Although colour is an important cue used by frugivores to find fruits, there are other important cues that could have been overlooked, such as fruit density, odours and texture (Dominy & Lucas 2001). Sanitjan and Chen (2009) found that ficus fruit colour and fruit size did not significantly influence the number of bird species, whereas habitat context appeared to influence the composition of visiting birds. Similarly, a plant–frugivore analysis at major river basins across Europe found that avian frugivore richness was more dependent on environmental factors than on fleshy fruited plant species richness (Marquez *et al.* 2004). Another example of habitat-specific effect is the distribution of polymorphic colour fruits of *Alepis flavida* in New Zealand. The mistletoes were mostly affected by habitat differences rather than avian frugivory (Bach & Kelly 2004a, b). Different levels of available light (caused by the thickness of canopy cover) among habitats have also influenced frugivore selection more than colour preferences (Cazetta *et al.* 2009). Most fruit-eating bird species do not specialise on the fruits of a particular plant species (Kissling *et al.* 2007). Instead, frugivorous bird species often treat fleshy fruited plant species as interchangeable (Herrera 2002; Zamora 2000). Plants with similar fruits might be used by a similar variety of frugivores, and subsequently might have similar distributions of dispersed seeds (Pizo 2002). Another example is the study of generalisation in pollination systems. Evolutionary biologists mostly preferred extreme specialisation in pollination systems, regarding generalisation as a rarity. Waser *et al.* (1996) argued that generalisation-the use of several plant species by a pollinator and of several pollinator species by a plant-appears to be the rule rather than the exception. This indicates that narrow specialisation rarely occurs and could not be expected on theoretical grounds. However, the Waser *et al.* (1996) findings are based on a majority of tropical forest examples, lacking evidence from other regions such as temperate forest.

Other fruit traits that might be equally important for frugivore selection are fruit size, fruit protection, fruit phenology, seed size, seed number and nutritional content of the fruits (Chen *et al.* 2004; Schaefer *et al.* 2003b; Willson *et al.* 1990; Willson *et al.* 1989; Gautierhion *et al.* 1985; Wheelwright & Janson 1985; Janson 1983; Knight & Siegfried 1983; Herrera 1982; Willson & Thompson 1982). Conspicuousness of fruit colours is not optimised specifically to bird vision, and there are other animals that disperse fruit in New Zealand, such as bats, lizards and weta. Each of these taxa sees and has different vision capabilities and eye structures (Burns 2006b; Duthie *et al.* 2006; Lord *et al.* 2002; Wotton 2002; Lord & Marshall 2001). There is evidence that fruit colour can explain differences in frugivore assemblage if the comparisons were based on interguild (e.g., when comparing primates and birds) (Lomascolo & Schaefer

2010; Voigt *et al.* 2004). Evidence for specific animal species selecting a specific fruit colour is almost unknown.

The preference analyses we performed are based on the avian eye model, and they therefore provide a more accurate result than previous studies (Burns *et al.* 2009). We acknowledge that our correlation analysis only involves the chromatic component, which allows the calculation of distances in tetrahedral colour space, whereas the achromatic component could not allow us to emulate the same procedure. There is also the possibility of a spatio-temporal scale effect, where a larger dataset and different taxonomic comparisons could produce different results (Burns 2004). With a refined null model calculation and the inclusion of the achromatic component, our results provide a more complete understanding of the dynamics of fruit and leaf colour.

In conclusion, we found no evidence of colour constraints between fruits and leaves based on the fruit-foliage hypothesis. There is also no support for the frugivore specificity hypothesis. Colour alone does not exclusively affect the interaction between plants and animals. We suggest that analysis of colour (both chromatic and achromatic) along with other fruit traits might produce different results.

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**Appendix 1**
Avian Eye Model

Material and Methods: Equations
Spectral analyses were performed using a USB Ocean Optics 2000 spectroradiometer and a Xenon Pulse X2 lamp light source. An object's reflectance properties were measured as the proportion of a diffuse reflectance standard (white standard). The fibre optics probe was mounted inside a matte black plastic tube to exclude ambient light. The distance between each object and the probe was fixed at 1 cm. The angles of illumination and reflection were fixed at 45° to minimise glare. Spectra were calculated at 5 nm intervals from 300 to 700 nm with SpectraSuite software. Irradiance was measured with a cosine-corrected sensor and a D65 (normal daylight) light bulb as a reference.

We quantified the appearance of leaves using the contrast comparison method, which follows simple colour pattern measures related to photon capture (Endler & Mielke 2005; Vorobyev *et al.* 1998). A detailed explanation of the mathematical formulation model is given elsewhere (Vorobyev *et al.* 1998; Osorio & Vorobyev 1996), but the following formulae suffice to predict the discriminability of any two of spectra, provided only that receptor spectral sensitivities and noise can be estimated. For an eye with $n$ spectral classes of photoreceptor viewing a surface with a reflectance spectrum, $S(\lambda)$, receptor quantum catches are given by:

$$Q_i = \int R_i(\lambda)S(\lambda)I(\lambda)d\lambda \tag{1}$$

where $\lambda$ denotes wavelength, $i = 1; 2; \ldots; n$; $Q_i$ is the quantum catch of receptor $i$, $R(\lambda)$ spectral sensitivity of receptor $i$, $I(\lambda)$ the spectrum of light entering the eye, and integration is over the visible spectrum. The $R(\lambda)$ values were obtained from Endler and Mielke (2005) for both $U$ and $V$ type eyes. To take into account receptor adaptation, receptor quantum catches are normalised to the background to give a value:

$$q_i = k_i Q_i \tag{2}$$

The coefficients $k_i$ describe the von Kries transformation, and they are chosen so that the quantum catches for adapting background is constant:

$$k_i = 1/\int I(\lambda)R_i(\lambda) \tag{3}$$

Let $f_i$ be the signal of receptor mechanism $i$, and the $\Delta f_i$ be the differences of the signals in receptor mechanisms between the stimuli. The coded quantum catches are relative rather than absolute values (according to Weber's law), thus:

$$\Delta f_i = \Delta q_i / q_i \tag{4}$$

where $\Delta q_i$ denotes the differences in the quantum catch between the stimuli. The integration of the Weber-Fechner law gives the signal of the receptor channel that is proportional to the logarithm of the quantum catch with Endler and Mielke's (2005) modification:

$$f_i = \ln(q_i) \tag{5}$$

The equation can be simplified as follows (when comparing chromatic contrast between spectra *a* and spectra *b*):

$$\Delta f_i = \ln(q_i a) - \ln(q_i b) = \ln(q_i a / q_i b) \tag{6}$$

Receptor noise is described by the signal-to-noise ratio, or by its inverse, the Weber fraction. The Weber fraction is calculated independent of intensity (independent of number of absorbed quanta), thus:

$$\omega_i = v_i / \sqrt{\eta_i} \tag{7}$$

where $v_i$ is the noise-to-signal ratio of a single cone [in this study, we used 0.05, as suggested by Endler and Mielke (2005) and Schaefer *et al.* (2007)]. $\eta_i$ refers to the number of receptor cells of type *i* within the receptive field (Endler & Mielke 2005).

We then proceed to calculate and compare two colour patches by measuring the chromatic differences (Endler & Mielke 2005; Vorobyev *et al.* 1998). The following equation is from Vorobyev and Osorio 1998:

$$\Delta S = \sqrt{\frac{\begin{array}{l}(\omega 1 \omega 2)^2 (\Delta f 4 - \Delta f 3)^2 + (\omega 1 \omega 3)^2 (\Delta f 4 - \Delta f 2)^2 + \\ (\omega 1 \omega 4)^2 (\Delta f 3 - \Delta f 2)^2 + (\omega 1 \omega 3)^2 (\Delta f 4 - \Delta f 1)^2 + \\ (\omega 2 \omega 4)^2 (\Delta f 3 - \Delta f 1)^2 + (\omega 3 \omega 4)^2 (\Delta f 2 - \Delta f 1)^2 +\end{array}}{((\omega 1 \omega 2 \omega 4)^2 + (\omega 1 \omega 3 \omega 4)^2 + (\omega 2 \omega 3 \omega 4)^2 + (\omega 1 \omega 2 \omega 3)^2)}} \tag{8}$$

Colour is defined as a point in a perceptual space whose co-ordinate axes represent quantum catches of receptors (Poirson & Wandell 1990). The discriminability of any two colours is described by the "distance," ΔS, between them in JND units. A colour patch with a JND value of more than 1 is at the threshold of discrimination from the background. Increasing JND values indicate increasing ease of distinction (e.g., from a larger distance), whereas values less than 1 JND are not discriminated. Achromatic (brightness) contrasts are calculated similarly:

$$\Delta S = |\Delta f_i / \omega_D| \tag{9}$$

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However, the exact working nature of achromatic signals in birds is still poorly understood (Hart 2001; Osorio *et al*. 1999; Campenhausen & Kirschfeld 1998). Double cones have a broad spectral sensitivity, which overlaps both long and medium wavelength-sensitive cones and are used in achromatic signal processing (non-colour based tasks) (Cuthill 2006; Jones & Osorio 2004; Hart *et al*. 2000). $\omega_D$ is therefore regarded as the value of the double cones. Because $\omega_D$ is the same for all targets, it does not affect relative achromatic contrasts. The only available double cone receptor data sensitivity is based on *L. lutea,* and the $\omega_D$ is valued at 0.05.