

1 **Adaptation of flower and fruit colours to multiple, distinct mutualists**

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16 SUMMARY

- 17 • Communication in plant-animal mutualisms frequently involves multiple perceivers. A
18 fundamental uncertainty is whether and how species adapt to communicate with
19 groups of mutualists having distinct sensory abilities.
- 20 • We quantified the colour conspicuousness of flowers and fruits originating from one
21 European and two South-American plant communities, using visual models of
22 pollinators (bee and fly) and seed dispersers (bird, primate and marten).
- 23 • We show that flowers are more conspicuous than fruits to pollinators, and the reverse
24 to seed dispersers. In addition, flowers are more conspicuous to pollinators than to
25 seed dispersers and the reverse for fruits. Thus, despite marked differences in the
26 visual systems of mutualists, flower and fruit colours have evolved to attract multiple,
27 distinct mutualists but not unintended perceivers. We showed that this adaptation is
28 facilitated by a limited correlation between flower and fruit colours, and by the fact
29 that colour signals as coded at the photoreceptor level are more similar within than
30 between functional groups (pollinators, seed dispersers).
- 31 • Overall, these results provide the first quantitative demonstration that flower and fruit
32 colours are adaptations allowing plants to communicate simultaneously with distinct
33 groups of mutualists.

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35 **Keywords:** fruit, flower, colour, pollination, seed dispersal, mutualism, community,

36 stimulation landscape.

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39 INTRODUCTION

40 Pollination and seed dispersal mutualisms form complex interaction networks potentially
41 involving dozens of species (Waser *et al.*, 1996; Bascombe & Jordano, 2007). In such
42 mutualistic networks, communicative traits such as colour and odour signals in flowers and
43 fruits generally undergo multiple selective pressures (Schaefer & Ruxton, 2011). Two main
44 factors are then expected to shape the evolution of plant signals: the convergence (vs.
45 conflict) of selective pressures, which is mainly determined by the degree of similarity in the
46 sensory perception of multiple perceivers (Campell & Aldridge, 2006; Lomáscolo *et al.*,
47 2010; Lomáscolo & Schaefer, 2010); and the extent to which intrinsic mechanisms such as
48 the physicochemical nature of traits, pleiotropy and genetic correlations, and selection by
49 other players constrain evolvability (Hansen, 2003). These two factors have been scarcely
50 considered together; thus it is still an open question whether and how plant signals are
51 adapted to communicate to multiple, distinct mutualists.

52 Different properties of plant signals can be selected by pollinators and seed dispersers.
53 Colour signals, for example, can be selected for the biochemical (e.g., as antioxidants;
54 Schaefer *et al.*, 2008) or visual properties of pigments (Schmidt *et al.*, 2004).
55 Conspicuousness is an important visual property of flower and fruit colours as it can be an
56 adaptation improving plant dispersal. For instance, the intensity of the colour contrast
57 between the background and artificial flowers and fruits (our definition of conspicuousness
58 hereafter) is negatively correlated with search time in bumblebees (Spaethe *et al.*, 2001) and
59 positively correlated with visitation rate in seed dispersing birds, respectively (Cazetta *et al.*,
60 2009). Yet, because these results stem from experiments on either a single species or a single
61 type of perceiver, a major unknown in plant-animal communication is whether flower and
62 fruit colours can be simultaneously conspicuous to several mutualists.

63 Theoretically, several factors should limit simultaneous adaptation of flower and fruit
64 colours to the visual system of several mutualist agents. First, pollen and seeds are most
65 frequently dispersed by animals with distinct and marked differences in their visual system.
66 For example, some pollinating flies are likely tetrachromatic (i.e. use four types of
67 photoreceptors for colour vision; for details, see in Methods S1 in Supplementary
68 Information), whereas bees are trichromatic (Briscoe & Chittka, 2001). Similarly, seed
69 dispersing birds are tetrachromatic, whereas primates are either dichromatic or trichromatic
70 and other seed dispersing mammals such as foxes and martens are dichromatic (Osorio &
71 Vorobyev, 2005). If differences in the number of photoreceptor types translate into perceptual
72 differences, colour signals would have to simultaneously adapt to distinct visual systems.
73 Second, increasing conspicuousness to several mutualists simultaneously increases
74 conspicuousness to nectar/pollen robbers and pulp/seed predators, i.e. to antagonists already
75 known to perceive and select colour signals. Indeed, physicochemical mechanisms underlying
76 colour stimuli cause them to be characterised by continuous, smoothly-shaped reflectance
77 spectra (Jaaskelainen *et al.*, 1990). Contrary to other stimuli like odours, colour stimuli cannot
78 exhibit peaks of stimulation that are sharply tuned to specific receptor sensitivities. Rather,
79 flower and fruit colours have necessarily broad reflectance spectra, possibly also in order to
80 simultaneously stimulate different visual systems. However, given that visual systems
81 processing colour stimuli invariably consist of a limited number of different types of
82 photoreceptors with broad and overlapping sensitivities (van Hateren, 1993), broad-band
83 reflectance spectra stimulating the visual system of intended perceivers would also stimulate
84 that of unintended perceivers. Last, the pleiotropic nature of genes coding for colour traits and
85 correlations between genes involved in flower and fruit colouration could further limit
86 separate adaptation to pollinators and seed dispersers if these have differing colour perception
87 (Strauss & Whittall, 2006).

88 We here assess whether adaptations to distinct mutualists occur in flower and fruit
89 colouration. Using psychophysical models of colour vision, we estimated the conspicuousness
90 of flower and fruit colours to pollinators (bee and fly) and to seed dispersers (bird, primate
91 and marten). Adaptation is expected to occur if flowers are more conspicuous to pollinators
92 than fruits are, and likewise for fruits and seed dispersers relative to flowers. This condition
93 may be facilitated or hampered depending on pleiotropic factors and genetic correlations
94 between flower and fruit colouration. We thus analysed the influence of correlation between
95 flower and fruit colouration on conspicuousness at different time scales. In addition, selection
96 by mutualists for increased conspicuousness should generate differential conspicuousness to
97 mutualists and to non-mutualists as a signature of adaptation; provided that these two groups
98 have different perception of colours. We thus tested whether conspicuousness is higher to
99 mutualists than to non-mutualists, and we investigated the degree of perceptual similarities
100 among mutualists and between mutualists and non-mutualists. More precisely, we used a
101 modelling approach to evaluate how differences in the number and sensitivity of
102 photoreceptor types translate into photoreceptor signals. Evidencing that flowers are both
103 more conspicuous to pollinators than fruits are and more conspicuous to pollinators than to
104 seed dispersers (and likewise for fruits) would represent the first quantitative demonstration
105 that flower and fruit colours are in general adapted to the eyes of distinct types of perceivers
106 within diversified mutualisms.

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109 MATERIAL AND METHODS

110 *Studied plants and animals*

111 The data on flower and fruit colouration come from one European and two South American
112 plant communities. The colouration of both flowers and fleshy fruits of 102 European species

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113 belonging to 28 plant families were measured between 2007 and 2012. Most of these species
114 were collected in Mediterranean scrublands in southern Spain (see Valido *et al.*, 2011) and a
115 few additional species were collected in the botanical garden of the University of Freiburg,
116 Germany. For the few flowers that appeared multi-coloured to human eyes, we considered the
117 dominant colour only. In southern Spain, bees and flies are the two main groups of pollinators
118 (Herrera, 1988), whereas birds and mammals such as foxes and martens are the main seed
119 dispersers for fleshy-fruited plants (Herrera, 1995). Furthermore, Barbary macaques (*Macaca*
120 *sylvanus*) were relatively common in Spain until the last glaciation (<0.1 Ma; Valverde,
121 1967). Based on the diet of extant individuals from Morocco (El Alami & Chait, 2012), we
122 further treated the Barbary macaque as a likely seed disperser of the Spanish plant community
123 in the past.

124 In South America the colouration of fruits from 111 species (45 families) was
125 measured in 2006 in Ilha do Cardoso, southern Brazil. In this subtropical island, birds are the
126 main seed dispersers, and primates contribute to the seed dispersal of some of the studied
127 plants (Cazetta *et al.*, 2012). In 2009 we measured floral colouration in 67 species (23
128 families) in the coastal community of Los Molles, Northern Chile, where bees and flies are
129 the main pollinators and only one hummingbird species occurs (Rodrigo Medel, *et al.*,
130 unpubl. data). For ten species, flowers exhibited more than one colour in approximately equal
131 proportion. We measured these colours separately. Colour data were not available for flowers
132 in Ilha do Cardoso and for fruits in Los Molles.

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134 *Colour measurements*

135 We measured the reflectance spectra of flowers, mature fleshy fruits and leaves gently
136 detached from the plants using an Ocean Optic USB2000 or an Avantes 2048 spectrometer
137 following the procedure described in Schaefer *et al.* (2007). For each plant structure of each

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138 species, we averaged replicated measurements from 5-20 items collected from different
139 individuals. We defined leaf colouration as the background against which flowers and fruits
140 are perceived by mutualists. Within a given community, we then used the same, averaged leaf
141 colouration for all species because fruit colours are not adapted to be conspicuous towards
142 their own, species-specific foliage (Schaefer *et al.*, 2007), and because the variation among
143 leaf colours is considerably limited compared to the variation among fruit or flower colours
144 when viewed by pollen and seed dispersers (e.g., Chittka, 1997; Regan *et al.*, 2001).

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146 *Modelling conspicuousness*

147 Colour conspicuousness was calculated as the distance between the signalling stimulus
148 (flower or fruit) and the background (leaf) locations in a colour space, which is a graphical
149 representation of how colour stimuli appear to the eye of a given perceiver. We estimated the
150 conspicuousness of Spanish flowers and fruits in six different colour spaces describing the
151 visual systems of honeybees, hoverflies, macaques, martens and birds (two types of visual
152 systems; for details, see Methods S1). The conspicuousness of Brazilian fruits was measured
153 within the colour space of birds (two types) and New-World primates. Because most New-
154 World primates exhibit polymorphism at an X-chromosome opsin gene, six visual systems
155 (either dichromatic or trichromatic) can theoretically be found within the same population
156 (Jacobs, 2008). In addition, photoreceptor sensitivities differ between Callitrichidae (e.g.,
157 marmosets, tamarins) and Cebidae (*Cebus*, squirrel monkeys) families, leading to twelve
158 possible visual systems in polymorphic New-World primates (Jacobs, 2008). We included
159 nine of these twelve systems in our analyses because three of them were almost redundant
160 (Methods S1). The conspicuousness of Chilean flowers was modelled to the eyes of bees and
161 hoverflies using the same data as for the Spanish flowers. Overall, we investigated colour

162 conspicuousness to five groups of perceivers (bees, flies, martens, primates and birds)
163 characterized by fifteen distinct visual systems.

164 We used two approaches to model colour spaces and to calculate conspicuousness.
165 First, we applied the classical Receptor Noise Limited (RNL) model of colour vision
166 (Vorobyev & Osorio, 1998; Methods S1). The RNL model was originally developed to
167 evaluate small perceptual differences in the colour space, i.e. differences close to the detection
168 threshold, but the model has also been successfully applied to estimate larger differences
169 (e.g., in honeybees: Hempel *et al.*, 2001; in birds: Stobbe & Schaefer, 2008; Cazetta *et al.*,
170 2009). In the RNL model, one unit of perceptual distance corresponds to one Just Noticeable
171 Difference (JND). Previously, we argued that colour conspicuousness as measured by
172 traditional psychophysical models of colour vision, such as the RNL model, cannot be
173 compared directly among species (for details, see Renoult *et al.*, 2013). Thus, in a second
174 approach we used the method of the stimulation landscape (Stimuland) that standardises
175 values of conspicuousness (Renoult *et al.*, 2013). A stimulation landscape consists of a
176 spectral space (the same for the fifteen landscapes, i.e. one for each visual system), which is a
177 six-dimensional space describing variation in reflectance spectra, plus one dimension (unique
178 to each landscape) indicating the conspicuousness value for each spectrum (see Methods S1).
179 The colour space used in the stimulation landscape was a chromaticity diagram extracted
180 from the photoreceptor contrast space, which is the multidimensional space describing for
181 each photoreceptor type the ratio between the quantum catch associated with the signalling
182 stimulus and that associated with background stimulus (Kelber *et al.*, 2003). Here,
183 conspicuousness is evaluated as the Euclidean distance between the stimulus and the centre of
184 the diagram. We then randomly resampled 10^5 times each of the six variables of the spectral
185 space to generate reference sets of reflectance spectra and conspicuousness values.

186 Conspicuousness was eventually standardised (within the interval [0;1]) using the cumulative
187 distribution of reference conspicuousness values (Methods S1).

188

189 *Statistical analyses*

190 We studied the adaptation of flower and fruit colours to the eyes of their mutualistic agents
191 using generalised linear mixed models implemented in the R package MCMCglmm
192 (Hadfield, 2010; R Development Core Team, 2011). The three communities were analysed
193 separately and in combination (pooling flowers from Spain and Chile, and fruits from Spain
194 and Brazil, respectively). The response variable was the conspicuousness, expressed either in
195 JNDs (RNL model) or in standardised unit (Stimuland), of flower and fruit colours modelled
196 according to the visual systems of the perceivers present in a given community. For the
197 combined dataset, we considered the visual systems of those animals that occurred in all three
198 communities: bees, flies, birds and trichromatic primates. For the latter visual system, we
199 analysed conspicuousness to Macaques because their photoreceptor sensitivities are close to
200 that of Cebidae from the New World (for details, Methods S1). Explanatory variables included
201 the number of photoreceptor types (di-, tri- or tetrachromatic), the dispersal service provided
202 by the perceiver (pollinator or seed disperser) and, for the Spanish dataset that included both
203 flower and fruit spectra, the reproductive structure (flower or fruit) and interactions between
204 plant structure and each of the other two fixed factors. We added a random effect term to
205 account for the non-independency between values of conspicuousness calculated with a given
206 visual system. For the Spanish data, we further compared this model to two other models
207 coding either plant species or the full plant phylogeny (see Methods S2) in a second random
208 effect nested with the perceiver group. By accounting for the non-independency of
209 colouration among plant structures within taxa, these two models allowed investigating the
210 influence of pleiotropy or genetic correlations in flower and fruit colouration. We specified a
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211 gamma distribution of the response variable with identity link function for all models. Models
212 were fitted with $3 \cdot 10^6$ iterations, discarding the first million and sampling every 200
213 iterations. We used flat uninformative priors with a uniform low degree of belief across all
214 parameters. Models were compared based on the Deviance Information Criterion (DIC;
215 Hadfield, 2010).

216 In order to study how differences in the number and sensitivity of photoreceptor types
217 translate into differences in photoreceptor signals, we assessed the difference in shape
218 between the fifteen standardised stimulation landscapes. This was achieved by calculating a
219 canonical distance matrix between visual systems from the standardised conspicuousness
220 corresponding to the 10^5 colour spectra randomly sampled in the spectral space. This distance
221 matrix was used to build a tree by hierarchical clustering with the average method using R (R
222 Development Core Team, 2011). The tree describes the relationships between visual systems
223 based on their similarities in colour signals coded at the photoreceptor level.

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226 RESULTS

227 *Correlations between flower and fruit colours*

228 In the Spanish community, the models discounting the correlation between flower and fruit
229 colouration within taxa had a markedly poorer fit ($DIC_{RNL} = 7705$; $DIC_{stimuland} = -884$) than that
230 of models accounting for such a correlation. Among the latter, the models coding plant
231 species ($DIC_{RNL} = 7626$; $DIC_{stimuland} = -1098$) was better than the model coding the full plant
232 phylogeny ($DIC_{RNL} = 7629$; $DIC_{stimuland} = -1086$). Fruit and flower colouration within the same
233 species are thus not independent; though, the shared ancestry with more distant taxa
234 (congeneric and confamilial species) does not affect the strength of this correlation for the

235 species set studied. Only models coding for plant species are considered in the following
236 analyses of the Spanish data.

237

238 *Comparison between flower and fruit conspicuousness*

239 Using either RNL models or stimulation landscapes, colour conspicuousness was significantly
240 influenced by the interaction between disperser (pollinators or seed dispersers) and the
241 reproductive structure of the plant (flower or fruit) in both the combined (e.g., $\beta_{\text{RNL}} = 1.01$; p
242 < 0.001 ; Table 1a) and the Spanish datasets (e.g., $\beta_{\text{RNL}} = 1.28$; $p < 0.001$; Table 1b; no
243 interaction term in Brazilian and Chilean datasets). Specifically, flowers were more
244 conspicuous to pollinators than fruits were with specified factor contrasts (e.g., combined
245 dataset: $\beta_{\text{RNL}} = 1.13$; $p < 0.001$; Fig. 1a-d), and fruits were more conspicuous to seed
246 dispersers than flowers were (e.g., combined dataset: $\beta_{\text{RNL}} = -0.52$; $p < 0.001$).

247 Based on stimulation landscapes, in all analyses we further found that flowers were
248 more conspicuous to pollen dispersers than to seed dispersers (e.g., with Chilean data:
249 $\beta_{\text{stimuland}} = -0.59$; $p < 0.001$; Table 1d), and the reverse for fruits (e.g., with Brazilian data:
250 $\beta_{\text{stimuland}} = 0.34$; $p = 0.005$; Table 1c). This result did not hold with RNL models except if
251 excluding birds (results not shown). However, given that comparing large perceptual
252 distances measured with RNL models across species leads to unreliable results (Renoult *et al.*,
253 2013), we propose that flower and fruit colours are more salient to their respective mutualists
254 than they are to non-mutualists.

255 For a given plant structure there was no effect of the number of photoreceptor types on
256 conspicuousness in any visual model or dataset. This is attested by the lack of significance of
257 the interaction term between photoreceptor number and plant structure in the overall and
258 Spanish datasets (Table 1a,b), and of the simple effect term of photoreceptor number with the

259 Chilean and Brazilian data (Table 1c,d). Thus, adaptations of flower and fruit colours appear
260 independent of the number of photoreceptor types used for colour vision.

261

262 *Comparison between stimulation landscapes*

263 Comparing the standardised conspicuousness of simulated colour spectra revealed substantial
264 variation in the shape of stimulation landscapes among perceivers that provide a similar
265 service of dispersion. This is shown by the terminal branches on the tree of shape similarities,
266 which are different from zero (Fig. 2). Shape similarities were not explained by the number of
267 photoreceptor types used to process colour stimuli: e.g., fly and bee stimulation landscapes
268 are clustered with maximal bootstrap support. Rather, the clustering of fly and bee landscapes
269 on the one hand, and of primates, birds and dichromatic mammals on the other hand indicates
270 that dispersal service or the phylogeny of animals can determine similarities in stimulation
271 landscapes.

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274 **DISCUSSION**

275 Most studies on colour signalling in plant dispersal mutualisms have focused on interactions
276 between plants and a specific pollinator and seed disperser (Chittka & Menzel, 1992;
277 Lomáscolo *et al.*, 2010; Lomáscolo & Schaefer, 2010). These studies have contributed to
278 illuminate when and how one prevalent pollinator or seed disperser, with its specific visual
279 abilities, can drive plant colour evolution, or can contribute to structure communities by
280 sorting species according to their colouration (e.g., in flowers see Kevan, 1983; Gumbert *et*
281 *al.*, 1999; Arnold *et al.*, 2009; Dyer *et al.*, 2012; in fruits see Willson & Thompson, 1982;
282 Burns & Dalen, 2002; Schmidt *et al.*, 2004; Schaefer *et al.*, 2007; Cazetta *et al.*, 2012). Yet,
283 pollen and seeds are most frequently dispersed by multiple mutualists having differing

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284 sensory systems. In this study, we analysed whether and how flower and fruit colours adapt to
285 simultaneously communicate with these multiple, distinct dispersers. We showed that flowers
286 are more conspicuous than fruits to pollinators, and the reverse to seed dispersers. In addition,
287 despite marked differences in the visual systems among pollinating and among seed
288 dispersing species, flowers are more conspicuous to pollinators than to seed dispersers and the
289 reverse for fruits.

290

291 *Adaption to mutualists' visual systems*

292 In order to demonstrate adaptation to several mutualists, we first expected that flowers are
293 more conspicuous to pollinators than fruits are, and likewise for fruits and seed dispersers
294 relative to flowers. Our results unambiguously support this prediction. The differential
295 conspicuousness of flowers and fruits to a given perceiver is facilitated by the weak
296 phenotypic integration of colour traits among flowering and fruiting displays. Indeed, even
297 though we found evidence that correlations between flower and fruit colouration are a
298 widespread phenomenon within plant species of the Spanish community, we also showed that
299 there are no strong effects on deeper phylogenetic levels. Thus, genetic correlations and
300 pleiotropy do not appear to be a major constraint in the evolution of flower and fruit
301 colouration. This finding is certainly related to the high versatility of the biosynthetic
302 pathways of plant pigments: minor changes in regulating factors may have profound effects
303 on the resulting colouration (Rausher, 2008). Our result therefore support recent suggestions
304 that colour signals are not only highly evolvable in animals (Endler *et al.*, 2005) but also in
305 plants (Valido *et al.*, 2011; Stournaras *et al.*, 2013).

306 We were further expecting that conspicuousness of a given plant structure is higher to
307 mutualists than to non-mutualists. Again, results with standardised estimates of
308 conspicuousness matched this prediction. Overall, the finding that flower and fruit colours can

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309 stimulate similarly and more strongly (compared to unintended animals) the eyes of distinct
310 pollinators and seed dispersers indicate that these traits are likely signalling adaptations
311 targeted towards distinct plant dispersers.

312 The higher conspicuousness of a given plant structure to mutualists compared to non-
313 mutualists could originate from selection exerted by mutualists. Flower and fruit phenotypes
314 that are more conspicuous and thus attract more mutualists would have increased fitness,
315 generating differential conspicuousness to mutualists and non-mutualists over evolutionary
316 times. Two conditions for this mechanism to occur are that (i) mutualists exert convergent and
317 non-independent selective forces (Iwao & Rausher, 1997), i.e. they have similar perception of
318 colour signals, and (ii) mutualists and non-mutualists have different perception of colours
319 because any increase in conspicuousness to mutualists would otherwise indirectly increase
320 conspicuousness to non-mutualists, independently of whether non-mutualists select plant
321 colours or not. Supporting both conditions, we found that the stimulation landscape is more
322 similar among pollinators and among seed dispersers than between these two groups.
323 Importantly, we showed that functional groups of mutualists exerting similar selective forces
324 on plant colouration should not be defined according to the number of photoreceptor types but
325 according to the perceived similarities.

326 Selection decreasing conspicuousness to non-mutualists could also generate
327 differential conspicuousness to mutualists and to non-mutualists. Although many flower and
328 fruit antagonists are insects and vertebrates, respectively, various insects such as some
329 butterflies, wasps and bugs are also fruit antagonists consuming fruit pulp without dispersing
330 seeds while also serving as vectors for fruit-colonizing fungi (e.g., Tewksbury *et al.*, 2008);
331 and many primate and bird species consuming nectar have important deleterious effects for
332 flower reproduction (e.g., Riba-Hernandez & Stoner, 2005). In addition, plant signals could
333 have been shaped to limit detection by the least effective mutualists (Lau & Galloway, 2004).

334 For example, it is often assumed that red colouration in flowers pollinated by red-sensitive
335 birds has evolved because it reduces detection by bees that are both less effective as
336 pollinators and less sensitive to red (Rodríguez-Gironés & Santamaría, 2004). The current
337 data do not allow disentangling between selection increasing or decreasing conspicuousness,
338 but the observed differential conspicuousness of flower and fruit colours to intended and
339 unintended perceivers can result from a combination of both types of selective pressure.

340

341 *Adaptation through spectral tuning*

342 Although the perception of colours modelled through photoreceptor signals shares
343 commonalities among dispersers of a given functional group, it also shows substantial
344 differences (Fig. 2). Given the intrinsic constraints that prevent a narrow matching of
345 reflectance spectra with the sensory sensitivities of perceivers, we need to ask how colour
346 signals can be simultaneously tuned to the sensitivity of multiple visual systems. Previous
347 studies showed that minor stepwise changes in reflectance could determine the
348 conspicuousness of flower and fruit colour signals to a given disperser if these changes occur
349 in areas of heightened sensitivity of the perceiver (Chittka & Menzel, 1992; Schaefer *et al.*,
350 2007). Provided that bee and fly visual systems share wavelengths with heightened
351 sensitivity, such changes could explain how colours can simultaneously stimulate markedly
352 distinct visual systems.

353 A synthetic stimulation landscape in which the dimension indicating colour
354 conspicuousness is the sum of standardised conspicuousness to bees and flies identifies the
355 colours that best stimulate simultaneously the perception of the two pollinator groups. Figure
356 3 illustrates such a landscape and elucidates two important aspects of adaptation to diversified
357 assemblages of pollinators. First, there are several peaks in this synthetic landscape indicating
358 that different local optima exist in stimulating both flies and bees. If different colours attract

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359 simultaneously bees and flies, plants adapting to these insects do not necessarily converge on
360 the same colour signal; a result that can contribute to explaining the pronounced floral colour
361 diversity in angiosperms. Second and equally important, the fact that small variations in the
362 shape of reflectance spectra can lead to pronounced variations in the summed
363 conspicuousness has important implications for the evolution of adaptations in colour
364 signalling. It suggests that the costly trade-offs typically inherent to adapting to multiple
365 mutualistic partners may not be common in visual communication because small variations in
366 several colours can increase simultaneously the conspicuousness to both bees and flies. This
367 in combination with the result that different colours stimulate both bees and flies strongly may
368 contribute to explaining the ubiquity of diversified interactions in pollen dispersal mutualisms
369 (Waser *et al.*, 1996).

370 Major changes in conspicuousness resulting from minor spectral variations also
371 explain how flower and fruit colours could have reduced conspicuousness to antagonists or to
372 ineffective mutualists. In addition, signals reflecting most of the light at one extreme of the
373 visible light spectrum, to which only effective mutualists are sensitive, could also contribute
374 to stimulating mutualists more than non-mutualists. Supporting this mechanism, there were
375 more fruits than flowers in our datasets with a deeply saturated red colour that is highly
376 conspicuous to birds (Fig. S3).

377

378 *Adaptation, colour preferences and conspicuousness*

379 Studies investigating possible adaptations of plant colouration to animal dispersers analysed
380 how hues segregate to different groups of animals; which is an indirect approach to analyse
381 associations between plant colouration and colour selection (e.g., Gautier-Hion *et al.*, 1985;
382 Arnold *et al.*, 2009; Campbell *et al.*, 2010). While hues can be associated with specific groups
383 of dispersers, there is little support that this association is driven by animals' colour

384 preferences. Often, there is marked variation in colour preferences among species,
385 populations and even individuals (McCall & Primack, 1992) and preferences are even
386 transient within individuals (Willson, 1994). This variation arises because colour preferences
387 (both innate and learned) can be themselves adaptive, i.e. are shaped to facilitate recognition
388 of beneficial objects (Raine & Chittka, 2007; Palmer & Schloss, 2010), and are thus context-
389 dependent. For example, a flower can be profitable or not to a given pollinator depending on
390 competition with other pollinators (Chittka & Waser, 1997; Valido *et al.*, 2002), availability
391 of alternative plant resources (Ghazoul, 2004), and environmental as well as genetic factors
392 influencing the production of rewards by plants (Mitchell, 2004) or colour signalling (e.g.,
393 herbivores influencing frequency of colour morphs; Irwin *et al.*, 2003). Studying the
394 association between hues and groups of perceivers may thus not be optimal to evaluate the
395 adaptation of plant colouration to dispersers because a lack of association could be due to
396 grouping perceivers at the wrong level (typically at species level when preferences differ
397 between populations; Lazaro *et al.*, 2008), while a positive association could be driven by
398 adaptation in perceivers but not in signallers.

399 In contrast to colour preferences, the perception of colours as coded at the eye level is
400 much more stable across perceivers (Briscoe & Chittka, 2001; Osorio & Vorobyev, 2008).
401 Indeed, the number and sensitivities of photoreceptor types are most frequently adapted for
402 'general-purpose' vision within a given environment, which constrains adaptation to a specific
403 visual task (Osorio & Vorobyev, 2008). As a consequence, diversification in plant colouration
404 should post-date diversification of photoreceptors (Chittka, 1997), meaning that a match
405 between flower or fruit colours and dispersers' perception of colours most likely originate
406 from an adaptive tuning of plant colouration. This explains why those studies interested in
407 colour conspicuousness or colour diversity with regard to the discrimination abilities of
408 animal dispersers (two aspects of visual communication determined mainly at the eye level)

409 unambiguously support adaptation of flower and fruit colouration to animal mutualists (this
410 study; Chittka & Menzel, 1992; Lomáscolo *et al.*, 2010; Dyer *et al.*, 2012; Shrestha *et al.*,
411 2013). One exception is the study by Lomáscolo & Schaefer (2010). These authors found that,
412 although bird-eaten and primate-eaten fruits can be well discriminated by birds and primates
413 based on colouration, both types of fruits are more conspicuous to birds than to primates. This
414 and our own findings together suggest that, in general, the colouration of flowers and fruits is
415 adaptively conspicuous to pollinators and seed dispersers, respectively, but above a minimal
416 threshold of conspicuousness, different colours can be selected (there are several peaks of
417 simultaneous conspicuousness; see above) depending on local colour preferences of
418 mutualists or on factors unrelated to communication.

419

420 *Considerations*

421 We caution against generalizing our conclusions too widely. We selected the Spanish and the
422 two South-American communities in this study because the identity of the main pollen and
423 seed dispersers allowed a balanced design with trichromatic and tetrachromatic perceivers
424 within each type of dispersal service. These communities show a robust pattern of adaptation,
425 but cannot represent all possible interactions between plants and dispersers. For example,
426 birds can also contribute substantially to pollination, reptiles sometimes visit flowers and
427 fruits and insects can disperse seeds of fleshy fruits (Duthie *et al.*, 2006). While more studies
428 are clearly needed in order to assess whether our results and the suggested mechanisms of
429 adaptation apply to other communities and ecosystems, the concordant results between the
430 Spanish and the two South-American communities suggest that adaptation of flower and fruit
431 colours to multiple, distinct mutualists may be widespread.

432

433

434 CONCLUSION

435 Since Aristotle, it has been paradigmatically assumed that flower colours are adapted to
436 attract pollinators (Chittka, 1997; Lee, 2007). A growing body of literature has shown,
437 however, how interactions with multiple species such as those occurring between most plants
438 and animal dispersers translate into disparate selective pressures (Thompson, 2005;
439 Guimarães *et al.*, 2011). Owing to this fact, the degree of adaptations in generalised plant-
440 animal mutualisms is contentious (Waser *et al.*, 1996; Fenster *et al.*, 2004). Here, we showed
441 that subtle adjustments in colour stimuli allow broad-band colour stimuli to match broad-band
442 receptor sensitivities of multiple mutualists. These adjustments can have important perceptual
443 effects, allowing adaptation of flower and fruit colour signals. This study suggests that
444 adaptation to a specific set of mutualists can occur more frequently than currently
445 acknowledged in colour signalling, even in mega-diversified networks of mutualistic
446 interactions.

447

448

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458

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- 603
- 604

605 TABLES AND FIGURES

606 **Table 1.** Results of the generalised linear mixed-effect model with all flowers and fruits (a),

607 with Spanish flowers and fruits (b), with Brazilian fruits (c) and with Chilean flowers (d).

608 Each cell indicates results with the RNL model (left) and with the stimulation landscape

609 (right). The random part includes the visual system (in all models) and species (with Spanish

610 data). Results are provided with default factor contrasts.

Fixed factors	post. mean of			pMCMC
	β	lower-95%	upper-95%	
(a) intercept	1.72/1.76	1.48/1.52	2.07/2.01	<0.001/<0.001
plant structure	-0.26/-0.51	-0.39/-0.64	-0.12/-0.37	<0.001/<0.001
no. photoreceptor types	-0.15/0.09	-13.18/-0.38	11.26/0.53	NS/NS
dispersal service	0.11/-1.03	-9.18/-1.39	9.30/-0.65	NS/<0.001
structure x photoreceptor	0.19/-0.14	-0.02/-0.36	0.41/0.06	NS/NS
structure x service	1.01/1.43	0.79/1.20	1.23/1.66	<0.001/<0.001
(b) intercept	1.92/1.95	1.56/1.70	2.31/2.21	<0.001/<0.001
plant structure	-0.44/-0.66	-0.61/-0.82	-0.28/-0.51	<0.001/0.008
no. photoreceptor types	-0.16/0.37	-17.20/-0.03	15.30/1.04	NS/NS
dispersal service	-0.08/-1.24	-6.69/-1.68	6.67/-0.86	NS/<0.001
structure x photoreceptor	-0.22/-0.32	-0.65/-0.68	0.20/0.04	NS/NS
structure x service	1.28/1.67	0.99/1.41	1.56/1.94	<0.001/<0.001
(c) intercept	-1.92/0.97	-4.12/0.82	0.43/1.10	0.08/<0.001
no. photoreceptor types	-0.48/0.23	-4.18/0.02	3.38/0.48	NS/NS
dispersal service	0.88/0.34	-2.99/0.10	4.59/0.55	NS/0.005
(d) intercept	-1.84/1.12	-3.71/0.94	0.09/1.26	0.064/<0.001

no. photoreceptor types	-0.63/0.17	-3.73/-0.02	2.34/0.38	NS/NS
dispersal service	0.01/-0.59	-3.21/-0.85	3.02/-0.34	NS/<0.001

611

612 **Figure 1.** Comparison of colour conspicuousness of flowers and fruits to pollinators and seed
 613 dispersers. **(a,b)** All flowers and all fruits combined, **(c,d)** Spanish, **(e,f)** Brazilian and **(g,h)**
 614 Chilean data. Bar height indicates mean conspicuousness along with the standard error either
 615 in JNDs units, i.e. calculated with RNL models **(a,c,e,g)**, or in standardised conspicuousness
 616 estimated using stimulation landscapes **(b,d,f,h)**. A fully captioned version of panel **(e)** is
 617 provided in Figure S1a.

618

619 **Figure 2.** Tree of similarities among stimulation landscapes reconstructed by comparing
 620 standardised conspicuousness of the same 10^5 artificial colour stimuli. Numbers above basal
 621 branches indicate bootstrap values. A fully captioned version in provided in Figure S1b.

622

623 **Figure 3.** Stimulation landscape of simultaneous conspicuousness of fruit and flowers to fly
 624 and bee visual systems. The landscape was constructed by adding the standardised landscapes
 625 of the bee visual system to the landscape of the fly visual system. Only the first three principal
 626 components of the stimulus space are represented. The summed standardised conspicuousness
 627 varies within the interval $[0;2]$ and is unit-free: blue and red colours indicate spectra that are
 628 lowly or highly conspicuous to both bees and flies, respectively. Black points indicate pairs of
 629 spectra that are physically close –as shown by the physical proximity in the landscape and by
 630 the reflectance spectra given in insets (abscissa: wavelengths in nm; ordinate: reflectance)–
 631 but are perceptually different –as indicated by the colouration of the landscape and by the

27

632 values above spectra (indicating the exact value of summed standardised conspicuousness for
633 each reflectance spectra).

634

635 **SUPPLEMENTARY INFORMATION**

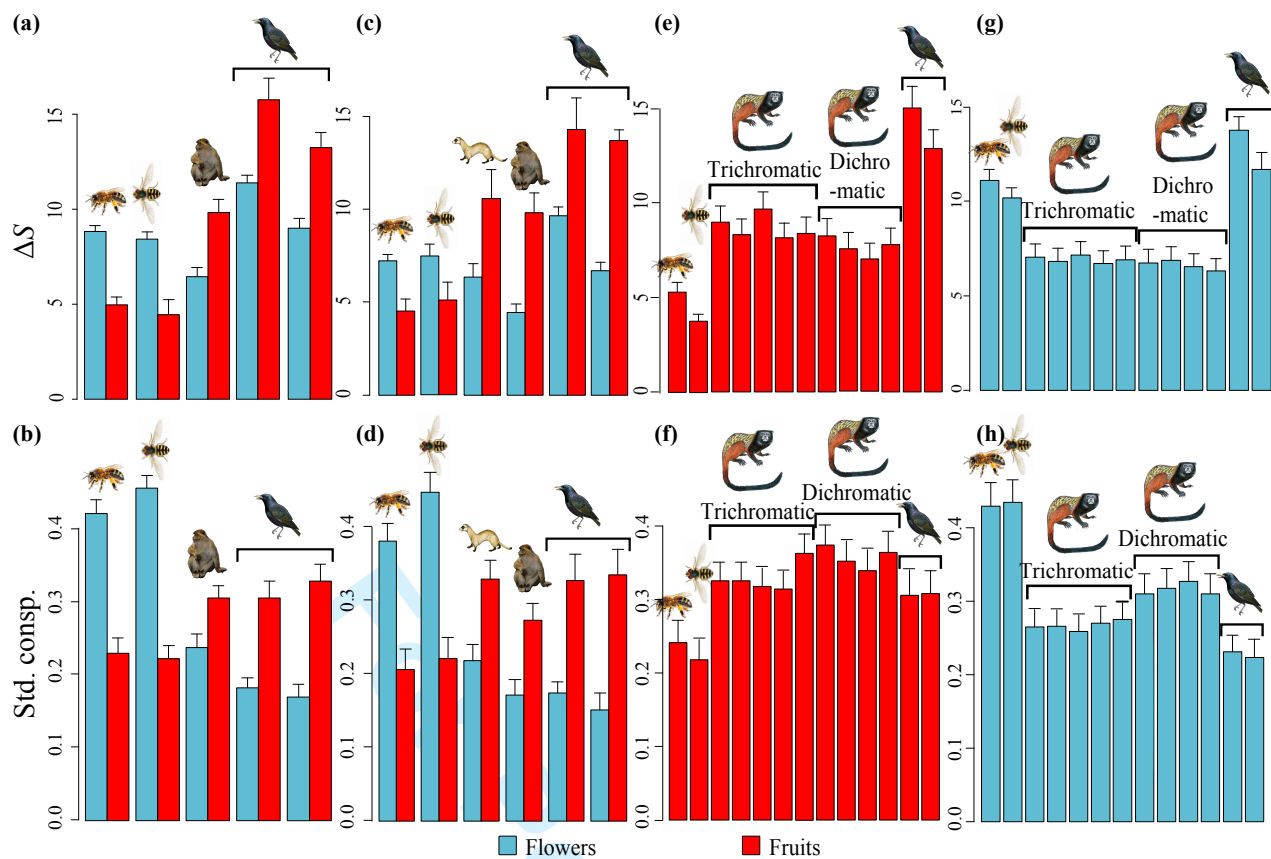
636 **Methods S1.** Supplementary methods for estimating conspicuousness.

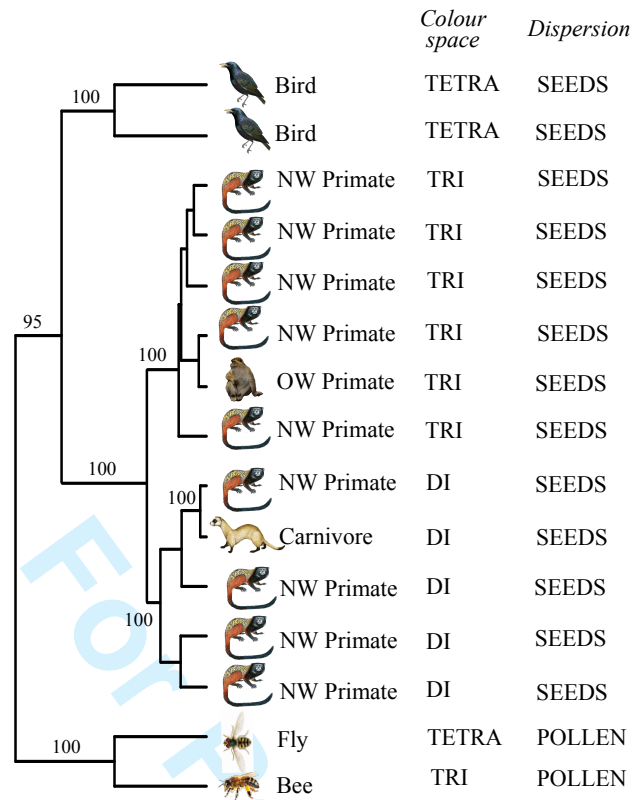
637 **Methods S2.** Phylogenetic relationships among the 102 Spanish plant species.

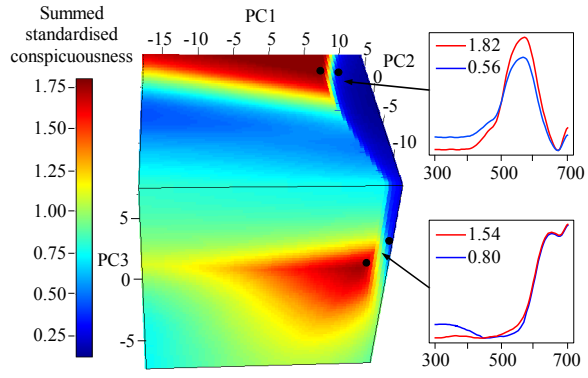
638 **Figure S1.** Fully captioned version of Figure 1e and Figure 2.

639 **Figure S2.** Distribution of fruit and flower colours in the bee, fly, macaque and bird colour

640 spaces.







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