

## Colour, design and reward: phenotypic integration of fleshy fruit displays

A. VALIDO\*, H. M. SCHAEFER† & P. JORDANO\*

\*Integrative Ecology Group, Estación Biológica de Doñana, CSIC, Sevilla, Spain

†Department Evolutionary Biology and Animal Ecology, Faculty of Biology, University of Freiburg, Freiburg, Germany

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### Abstract

The functional or structural linkage among traits [phenotypic integration (PI)] within complex structures can constrain the evolutionary response of individual traits. To analyse whether frugivores with distinct sensory ecology have shaped the patterns of fruit diversification differently, we compared PI values of fleshy fruits that are consumed by birds and mammals. We used phylogenetic comparative analyses of PI among 13 morphological, nutritional and visual fruit traits from 111 Mediterranean plant species. Results showed that morphological traits had higher PI values than nutritional and colour traits. Visual and nutritional traits show positive phylogenetic covariance, while negative covariation occurs between fruits size and nutrients. Importantly, fruits consumed by birds were relatively more integrated than fruits consumed partly or solely by mammals. Hence, we show that major groups of mutualistic frugivores can shape the covariance among some fruit traits differently and thereby influence fruit diversification.

### Introduction

A central interest of evolutionary biologists is to understand how natural selection drives the patterns of trait evolution and how adaptive changes may be constrained (e.g. Antonovics, 1976; Armbruster, 1991; Armbruster & Schwagerle, 1996; Pigliucci & Preston, 2004). Constraints limit or bias the evolutionary response of individual traits along certain paths (e.g. Berg, 1960; Gould & Lewontin, 1979; Schwenk & Wagner, 2004). This can be quantified by assessing the degree of phenotypic integration (PI), which is defined as the pattern and magnitude of covariation among sets of functionally related morphological traits (e.g. Olson & Miller, 1958; Wagner, 1984; Armbruster *et al.*, 1999; Pigliucci & Preston, 2004). The 'principle of coevolution of coexpressed traits' (West-Eberhard, 2003) articulates the idea of PI as a particularly important mechanism for maintaining the functionality of complex organs and structures.

*Correspondence:* Alfredo Valido, Integrative Ecology Group, Estación Biológica de Doñana, CSIC, C/Américo Vespucio s/n Isla de La Cartuja, 41092 Sevilla, Spain. Tel.: +34 954 466700; fax: +34 954 621125; e-mail: avalido@ebd.csic.es

When considering mutualistic interactions of animals and plants, whose outcomes pivot around complex structures such as flowers and fruits, a central question is to what extent the patterns of PI represent adaptations to the partner species (see examples in pollination studies in e.g. Murren, 2002; Herrera *et al.*, 2002; Pérez-Barrales *et al.*, 2007; Pérez *et al.*, 2007). Fleshy fruits (fruits hereafter) provide an excellent model for testing this question because they represent well-defined but phenotypically and functionally complex organs resulting from various combinations of visual, nutritional and morphological traits into a single, complex, anatomical structure. These sets of traits interact with the visual, tactile and gustatory senses of frugivorous animals. If these animals differ in their sensory ecology, they can select fruit traits differentially (e.g. Gautier-Hion *et al.*, 1985; Jordano, 2000; Lomáscolo *et al.*, 2010) potentially leading to different degrees of PI by favouring distinct combinations of traits. At the same time, the patterns of PI are influenced by internal processes like ontogeny because fruits are anatomically and morphologically complex organs originating from the flower. For example, the number of flowers and

ovaries translates into fruit design from simple to aggregate and multiple fruits, and the size of flower and ovary to fruit size (e.g. Primack, 1987; Rosati *et al.*, 2009). It is therefore unknown whether PI in fruits constrains the evolution of fruit diversification or whether it represents adaptations to distinct groups of animal frugivores.

Comparing PI among visual, morphological and nutritional fruit traits is interesting for several reasons. First, only few studies found evidences for adaptations to distinct seed dispersers in single (Jordano, 1995a; Lomáscolo & Schaefer, 2010) or co-occurring sets of fruit traits (Lomáscolo *et al.*, 2010). Second, visual fruit traits are expected to covary with nutritional fruit traits if they function as signals in plant–animal communication (Schaefer & Schmidt, 2004; Schaefer *et al.*, 2008), but this conjecture has not been examined using phylogenetically informed methods. Third, visual, morphological and nutritional fruit traits interact with the senses of dispersers in a hierarchical manner. Fruit colours attract frugivores from a distance, fruit morphology interacts with the tactile senses once a consumer decided to pick a fruit, and, finally, fruit chemistry interacts with taste and digestion if a fruit is swallowed (e.g. Jordano, 1987, 2000; Levey, 1987; Sallabanks, 1993; Izhaki, 2002). As such, distinct degrees of PI among these morphological, visual and nutrient trait sets will show the relative importance of each set for fruit consumption by animals.

Here, we analyse the covariance among 13 fruit traits in a large, representative, sample of Mediterranean fleshy-fruited plant species whose seeds are dispersed by two frugivore guilds (birds and mammals) that differ in sensory ecology. If the sensory ecology of frugivores influences the evolution of fruit traits, we predict that the degree of PI differs among frugivores. Conversely, similar covariance patterns of bird- and mammal-fruits would suggest PI is primarily contingent on genetic or developmental constraints. To account for phylogenetic influences on fruit traits, we tested the degree of PI on the covariation patterns of phylogenetically independent contrasts (PICs) of fruit traits.

Our specific objective is to test the hypothesis that the sensory ecology of frugivores influences the evolution of fruit displays. We therefore compare the degree of PI of visual, nutritional, and morphological fruit traits between fruits consumed by birds and mammals. Because frugivorous birds have better colour vision than frugivorous mammals and because birds are more restricted by gape width in the size of fruits that they can consume, we predicted specifically that the PI values of morphological and visual fruit traits are higher in fruits consumed by birds compared to fruits consumed by mammals. Our last objective was to test the hypothesis that the visual component of fruit displays can function as signal to animal frugivores. According to this hypothesis, we predicted that visual and nutritional fruit traits covary independently of plant phylogeny.

## Materials and methods

### Study system: fleshy-fruited plants of the Iberian Peninsula

We studied fruit traits in 111 plant species native to the Mediterranean phytogeographical and bioclimatic region of the Iberian Peninsula. We selected this area because it is the only one where quantitative information on morphological, nutritional and visual fruit traits is available for the majority of fruit species. Moreover, plant–frugivore interactions have been intensively studied and are well documented in this region, both by previous studies (see Appendix S1, for literature sources) and also by our own unpublished field observations. Based on this information, we classified plant species into three broad categories, fruits being consumed only by birds, by birds and mammals (mixed disperser assemblage), and only or largely by mammals (see Appendix S1). These categories have been used in previous studies (e.g. Janson, 1983; Knight & Siegfried, 1983; Debussche & Isenmann, 1989; Jordano, 1995a; Lomáscolo *et al.*, 2008, 2010) and aim to characterize the relative position of each species along a gradient between a strong reliance on avian seed dispersers to a strong reliance on mammals as seed dispersers. Thus, if frugivores influence fruit integration, plant species within each category should be more similar to each other than to species from other categories.

The taxonomic information and the regional distribution of each plant species were based on information available from the ANTHOS project from Real Jardín Botánico de Madrid (RJB, CSIC) (<http://www.anthos.es>). According to this plant database, our data set is representative for the Western Mediterranean Basin as it includes ~70% of all species, 93% of the genera ( $N = 64$ ) and 89% of the families ( $N = 34$ ) of the fleshy-fruited flora in this area.

### Fruit traits

We used 13 fruit variables for the analyses based on three criteria. We (i) selected species with reasonable complete data for the 13 traits to minimize missing values; (ii) dropped composite variables (e.g. relative yield of pulp, energy content per fruit, specific energy content of pulp) that have inherent redundancy with other simple variables; and (iii) eliminated variables especially prone to measurement error or inconsistency (e.g. pulp water content). We categorized fruit traits into three distinctive groups that address different sensory modes of vertebrate frugivores: (i) fruit morphology, including maximum fruit diameter (diam), fresh fruit mass (frfm), dry mass of pulp (pdm), dry mass of seeds (sdm) and number of seeds/fruit (seeds); (ii) pulp nutrient content, including the proportions of total lipids (lip), protein (pro), non-structural carbohydrates (nsc), ash and acid-detergent fibre (fib\_ac) on a dry mass basis of the pulp; and (iii)

fruit colour, including brightness (bright), hue and chroma (chrom). The complete data set is included in Table S1 (see also <http://dx.doi.org/10.5061/dryad.8011>).

Morphological, nutritional and colour fruit traits were compiled from both published sources (see references list in Table S1) and our own data (including 14 species for fruit morphology, 20 for pulp nutrient content and all species for colour traits; see Table S1). Morphological data for these species were measured on 40–80 fresh fruits from > 20 individual plants for each by species (but < 10 individuals for *Atropa baetica*, Solanaceae; *Crataegus laciniata*, Rosaceae; *Juniperus thurifera*, Cupressaceae). We used a higher number of fruits for analyses of nutritional contents to increase the dry mass of pulp necessary for the different analytical procedures. We only used data from the literature if all morphological and nutritional variables were obtained by means of similar analytical standard methods (according to those described in Jordano, 1995a). To calculate pulp dry mass, we dried fruits at 40 °C to constant weight during 2–4 days in a forced-air oven. The dried pulp samples were analysed for contents of total crude protein (*N* extraction by Kjeldahl method and with a conversion factor of 6.25), crude lipids (Soxhlet extraction), total nonstructural carbohydrates (calculated by difference), acid detergent fibre (according to van Soest procedures) and ash (incineration at 550 °C).

To characterize fruit colour for the 111 species, we measured the reflectance spectra of 20–40 ripe fruits collected from different individual plants. We used an Ocean Optics USB-2000 spectrometer and a Top Sensor System Deuterium-Halogen DH-2000 lamp as a standardized light source (DT-MINI-GS-2). Reflectance was measured as the proportion of a standard white reference tile (WS-1-SS; Ocean Optics, Duiven, The Netherlands). We used a coaxial fibre cable (QR-400-7-UV-VIS-BX; Ocean Optics) for all measurements and held the distance between the fruit sample and the measuring probe constant. The angle of illumination and reflection was fixed at 45°. Spectra data were processed with *SPECTRASUITE* software (version 10.4.11; Ocean Optics) and calculated in 5-nm-wide spectral intervals over the range of 300–700 nm. We thus incorporated the entire range of UV that is visible to birds as frugivores.

It is desirable to analyse fruit colour according to the visual system of fruit consumers. However, in our phylogenetically explicit analyses, colour traits need to be assigned unambiguously and thus independently of the distinct visual systems of birds and mammals. Hue, chroma and brightness provide such unambiguous notation for characterizing colours (Endler, 1990). They are therefore the most commonly used colour variables in comparative studies (Montgomerie, 2006). Total brightness [or intensity;  $R(\lambda)$ ] measures the cumulative sum of the light intensity reflected between 300 and 700 nm from a given surface ( $R_i$ ). Hue (or spectral shape) is measured as the wavelength of maximal reflection

[ $\lambda(R_{\max})$ ]. Chroma is a measure of colour saturation or spectral purity and is calculated as  $(R_{\max} - R_{\min})/R_{\text{average}}$ .

### Statistical analysis

Our statistical analysis of the data set (Table S1) is based on the Pearson product-moment correlation coefficients among all 13 fruit traits and also among traits in each of the three groups (morphology, five variables; nutrients, five variables; and colour, three variables). In the few cases of species with missing values, we used a pairwise deletion procedure to estimate the correlation coefficients. Instead of using the raw data, the analyses were based on the PICs of the fruit traits. This takes fully into account the phylogenetic information in the analysis of comparative data (e.g. Garland *et al.*, 1992). We used the conservative tree for angiosperm families based on Stevens (2001), with branches supported by bootstrap values <80% or those 'weakly supported' left as soft polytomies. We built the plant phylogeny using the online software *PHYLOMATIC* (Webb & Donoghue, 2002; <http://www.phylodiversity.net/phyloomatic/phyloomatic.html>). To resolve polytomies in some plant groups (*Vaccinium-Arbutus*, *Viburnum-Sambucus*, Rhamnaceae, *Prunus*), we used recently published molecular phylogenies and incorporated taxonomic information for *Asparagus* when molecular phylogenies were not available (see references list in Appendix S2). The remaining polytomies were randomly resolved for the comparative analyses (Paradis, 2006). Branch lengths proportional to time are currently not available for all our clade, and we opted to recode branch lengths to a constant value of 1.0, except for those introduced by resolving polytomies (using infinitesimal branch lengths). Simulations with equal branch lengths performed better than those with topological lengths based on Grafen's algorithm but inflate type I error rates (Purvis *et al.*, 1994; Ackerly, 2000). However, at  $N > 64$ , no effect on statistical power was detected when comparing results of analyses using the actual branch lengths on the true tree and those using equal branch lengths (Ackerly, 2000). Thus, we consider that the proposed analysis with soft polytomies and equal branch lengths in our tree of 111 species provides robust estimates of the correlation coefficients among PICs.

The final phylogenetic tree used for the analysis is presented in Fig. S1. PICs were estimated with library *ape* (Paradis, 2006) of the *R* package (R Development Core Team 2005), with additional analyses carried out with libraries *base*, *agricolae*, *ade4*, *boot*, *nlme* and *psy*. We use here the phylogenetic generalized least squares method for obtaining the correlations among contrasts (Paradis, 2006). It handles adequately the multichotomies in the tree and additionally relaxes the assumptions of equal variances and covariances equal to zero among characters. Briefly, each node is given a height equal to the number of tips minus one, and these values are

scaled by raising to the power of  $\rho$ , with  $\rho > 0$ ; the root is set to have height equal to 1. Under a Brownian model of trait evolution, the covariance between species  $i$  and  $j$  is given by  $v_{ij} = \sigma^2 T_a$ , where  $T_a$  is the distance between the root and the most recent shared ancestor of  $i$  and  $j$  and is the variance associated to the Brownian process. However, we use an Ornstein–Uhlenbeck model of character evolution (Paradis, 2006), whereas the covariance between two species is given as  $v_{ij} = \sigma^2 \exp(\rho * d_{ij})$ , where  $\sigma^2$  is similar to the variance of a Brownian motion process,  $\rho$  is a parameter specifying the rate of character divergence after speciation, and  $d_{ij}$  is the phylogenetic distance between the two species. We use function `corMartins` in library `ape` to estimate the expected evolutionary correlation to compare with the actual one. We estimate an associated AIC value and test of significance. All the correlations between contrasts were tested through the origin (Paradis, 2006) and were based on log-transformed values of the original variables.

For each variable in the data set, we estimated its degree of phylogenetic dependence, i.e. to what extent the phylogeny we used explains the pattern of character variation across species. We used a multivariate decomposition approach (Ollier *et al.*, 2006) that considers a distance matrix among species derived directly from the topology of the tree. The approach is related to the one used by Diniz-Filho *et al.* (1998). An orthonormal transformation of this matrix is obtained leading to its eigenvector decomposition; the positive eigenvectors extracted are regressed against the studied trait. The eigenvectors obtained from the phylogenetic distance matrix capture the tree structure in vector form, so that the test of phylogenetic dependence for a trait is simply the test of a linear model where the eigenvectors are predictor variables (Ollier *et al.*, 2006). The model yields an F test for the effect of phylogeny on trait variation; the associated  $R^2$  value is used to estimate the percentage of variation in trait values across species that is accounted for by the phylogenetic relations among species (as defined by the phylogenetic tree) (Paradis, 2006).

The degree of PI was estimated by the variance of the eigenvalues of the correlation matrix (Lande & Arnold, 1983; Wagner, 1984; Cheverud *et al.*, 1989),  $\text{var}(\lambda_i)$ ,  $i = 1, \dots, 13$ . A high variance among extracted eigenvalues shows that most of the phenotypic variation is accounted for by the first dimension, which indicates a strong overall correlation among variables. We corrected this value by subtracting the expected value under random covariation among traits; the rationale for this is that the number of species used for correlations of different sets of variables was different, and thus the correlation matrices were estimated on different sample sizes and number of variables (Wagner, 1984). This expected value for  $n$  traits measured for  $s$  species is  $(n - 1)/s$ . We also expressed the observed  $\text{var}(\lambda_i)$  as a percentage of its expected maximum (the total number of traits) (Wagner, 1984). Nonparametric bootstrap esti-

mates and confidence intervals for  $\text{var}(\lambda_i)$  were obtained by the percentile method (Efron & Tibshirani, 1993). We estimated the  $\text{var}(\lambda_i)$  values for the combinations of fruit types and frugivore groups. The corrected  $\text{var}(\lambda_i)$  value for a given matrix was considered significant if its 95% confidence interval did not include zero. Differences in  $\text{var}(\lambda_i)$  among matrices were considered significant if the associated 95% confidence intervals did not overlap.

To test whether the three groups of traits were independently associated in the correlation matrix, we used a quadratic assignment procedure against a hypothesis matrix specifying the expectation of no integration among fruit traits of different groups (Dow & Cheverud, 1985). The hypothesis matrix depicts a full pattern of integration among traits within each group (morphology, nutrients and colour) and no integration among traits in different groups. It specifies correlations equal to 1 for trait pairs included in the same group and correlations equal to 0 for traits not in the same group. We used a Mantel's test to assess the correlation between the observed matrices and the hypothesis matrix. The test is based on random permutations (Dow & Cheverud, 1985) so that a significant positive association between the observed and the hypothesis matrices would be indicative of an integration pattern restricted to traits of the same set, with no correlations among traits of different set, i.e. a hypothesis of independence of the morphology, nutrient and colour traits.

## Results

### Correlation patterns and integration

Significant phylogenetic pairwise correlations among fruit traits were detected in one-third of all possible correlations (Tables S2). These correlations persisted if we did not include phylogenetic information (TIP values; data not shown; Mantel's test TIP vs. PIC values,  $r = 0.824$ ,  $P < 0.0001$ ,  $N = 10\,000$  permutations).

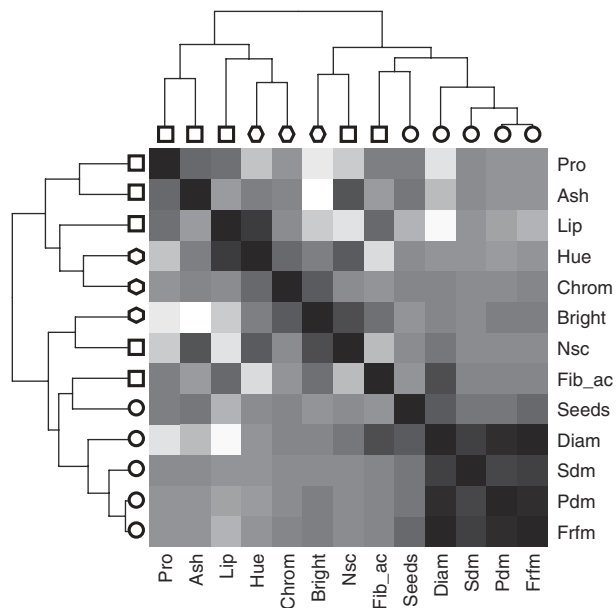
Consistent with our expectations, most traits within a set of traits (fruit morphology, nutrient content of pulp and colour) were strongly correlated, while most pairwise correlations among traits in different sets were nonsignificant. For example, almost all morphological traits were strongly correlated ( $P < 0.01$ ) except for the number of seeds with the dry mass of pulp and that of seeds. Likewise, nutritional traits were also correlated among each other ( $P < 0.05$  in most cases), except for the contents of ash and lipid, and fibre with protein and with ash ( $P > 0.10$ ). Concerning fruit colour, chroma was strongly correlated with the hue and brightness ( $P < 0.01$ ), but there was no correlation among hue and brightness ( $P = 0.235$ ). Moreover, it is important to note that practically all the significant negative correlations ( $P < 0.05$ ) were detected between traits belonging to different functional groups. For example, fruit diameter, fresh fruit mass and pulp dry mass correlated



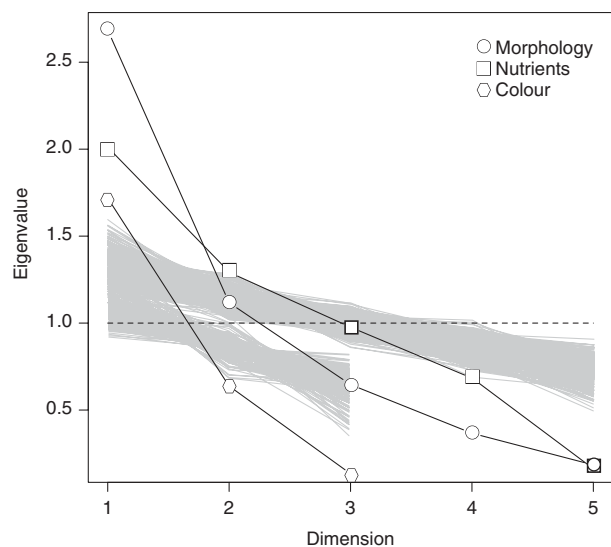
negatively ( $P \leq 0.05$ ) with the contents of lipids and proteins, while the contents of protein correlated negatively with brightness and nonsoluble carbohydrates, among others ( $P < 0.01$ ) (Table S2; Fig. 1).

Plotting traits according to their pattern of correlations to other fruit traits yielded clusters of highly integrated variables that roughly corresponded to the *a priori* grouping of traits into morphological, nutritional and colour sets according to sensory modes (Fig. 1). These clusters were very consistent when using the raw data (TIP; data not shown) and PICs. One distinct cluster consisted of morphological traits only. The other clusters contained both nutritional and visual traits (Fig. 1). These results indicate that hue and lipids as well as sugar and brightness are more tightly linked among each other throughout the evolutionary history of fruiting plants than they are linked to other traits within their functional group.

When all fruit traits were considered together, the integration value is relatively low ( $\text{var}(\lambda_i) = 1.67$ ; 12.8%). Overall, our data thus indicate that fruits are not strongly integrated across different traits that define fruit displays. When we derived the eigenvalues for the correlation matrices separately for each group of traits according to the sensory mode, all showed dominant



**Fig. 1** Heat-colour map for the correlation matrix of phylogenetically independent contrasts of all fruit traits (based on information shown in Table S2). Morphological (circles), pulp nutrient content (squares) and fruit colour (hexagons) traits were also grouped by a hierarchical cluster analysis of the correlation patterns, using Ward's minimum variance method. All fruit traits were intrinsically correlated within their group of traits. Nonstructural carbohydrates and lipids were significantly correlated with colour variables. The different shadows of grey indicate the correlation values, from high positive correlations (darker) to negative correlations (lighter).



**Fig. 2** Eigenvalues scree plot of the correlation matrix based on phylogenetically independent contrasts among fruit traits. The figure illustrates the sorted, decreasing, order of eigenvalues (abscissa) vs. the actual value of  $\lambda_i$  (ordinate). The grey lines illustrate bootstrapped values ( $N = 200$  runs), expected under a random pattern of correlations among traits.

eigenvalues higher than 1 (Fig. 2); again the pattern is very consistent between tip values (data not shown) and PICs correlations.

Consistent with the results of the clustering of correlation patterns, the values of PI differ between functional fruit trait groups (Table 1; Fig. 2). We found a clear gradient from high levels of integration in morphology (38.5%) to moderate levels of integration in nutrients (12.2%) and relatively lower for colour variables (8.7%). In all three groups of traits, a generally high percentage of phenotypic variation across species is explained by phylogeny (50.1–60.6%) (Table 1).

### Phenotypic integration and fruit-frugivore types

We assessed the relative PI values of morphological, nutritional and visual traits separately for plant species that differed in the main disperser type (bird, mixed or mammal). We observed the same overall trend of higher integration values in morphological traits compared to nutritional and visual traits independent of fruit and frugivore type (Fig. 3). Fruit morphology, nutrients and colour were more strongly integrated in fruits consumed by birds compared to fruits consumed by birds and mammals or by mammals only (Fig. 3).

### Discussion

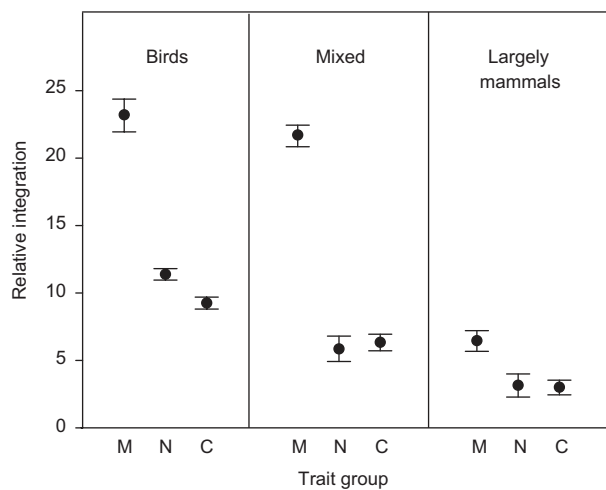
Fleshy fruits as anatomical structures show only a moderate level of overall PI among their morphological,

**Table 1** Summary of phenotypic integration statistics for the three groups of fruit traits (morphology, nutrients and colour) separately showing the numbers of traits measured for each group, the variance of the eigenvalues [ $\text{var}(\lambda_i)$ ], and the integration relative to the maximum expected. The mean percentage of variation explained by phylogeny and the level of significance for each trait is also indicated.

Trait group	No. of traits	PIC $\text{var}(\lambda_i)$	PIC, %	Mean % (range) variation explained by phylogeny	Significant phylogenetic effects
Morphology	5	1.92	38.5	60.6 (47.9–68.3)	diam*, frfm***, pdm**, sdm <sup>ns</sup> , seeds*
Nutrients	5	0.65	12.2	51.9 (38.2–62.7)	lip***, pro†, nsc <sup>ns</sup> , ash <sup>ns</sup> , fib_ac**
Colour	3	0.26	8.7	50.1 (42.1–58.2)	bright‡, hue <sup>ns</sup> , chrom***

PIC, phylogenetically independent contrast; diam, maximum fruit diameter; frfm, fresh fruit mass; pdm, dry mass of pulp; sdm, dry mass of seeds; seeds, number of seeds/fruit; lip, total lipids; pro, protein; nsc, nonstructural carbohydrates; fib\_ac, acid-detergent fibre; bright, brightness; chrom, chroma.

\* $P < 0.0001$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.01$ ; † $P < 0.05$ ; ‡ $P < 0.10$ ; ns, nonsignificant.



**Fig. 3** Plot of the relative phenotypic integration values for the three sets of fruit traits (M = morphology; N = nutrient; C = colour) with species grouped by the type of frugivorous vertebrates acting as seed dispersers. Plant species were classified according to their reliance on birds vs. mammals for seed dispersal (primarily dependent on birds or primarily dependent on mammals for fruit removal based on available information; see reference list in Table S1 and Appendix S1). The category 'mixed' includes species having both birds and mammals consuming the fruits.

nutritional and visual characteristics. However, we documented a considerable yet quite variable level of PI within these suites of traits. PI decreased from morphological to nutritional and visual traits. Visual and nutritional traits covaried consistently throughout the plant phylogeny supporting the hypothesis that visual cues can serve as reliable indicators of nutritional rewards in fleshy fruits. Importantly, we found that the relative integration of traits was associated to the major type of frugivore: Fruits consumed only by birds are consistently more integrated than fruits consumed by birds and mammals (mixed disperser coteries) and those consumed only (or largely) by mammals. This result supports the conclusion that major groups of frugivores vary in their

selective pressures on the structural pattern and magnitude of covariation of morphological, nutritional and visual fruit traits.

We will first discuss the patterns of covariance among fruit traits that might limit or channel fruit evolution throughout phylogenetic history. We limit our discussion to the covariation among those traits that are most closely linked to our hypotheses on the sensory ecology of frugivorous vertebrates. We will then highlight the potential role of vertebrate frugivores in shaping fruit traits.

### Fruit integration

We identified consistent patterns of PI among fruit traits using phylogenetically independent contrasts. A high integration level of phylogenetic contrasts implies conservatism not only in individual traits and their evolutionary trends but also in the evolutionary trends of covariation among traits during the diversification of fleshy-fruited plants. Phylogenetically consistent patterns of PI have also been recently reported in leaves, flowers and dry fruits as well as in the morphology of some animal organs (e.g. Ackerly & Donoghue, 1998; Pigliucci *et al.*, 1999; Marroig & Cheverud, 2001; Murren *et al.*, 2002; Baker & Wilkinson, 2003; Young & Badyaev, 2006; Pérez *et al.*, 2007).

Given that morphological traits are more integrated than nutritional and colour traits, we suggest that physiological, developmental and/or genetic constraints have stronger effects on fruit morphology. This is explicable because certain combinations of morphological traits are selected by optimal surface/volume ratio in fruits, while allometric effects also arise from developmental constraints originating from flower design, particularly from the shape and size of the gynoeceum (e.g. Primack, 1987; Jordano, 1995a; Rosati *et al.*, 2009). Our analysis further revealed negative correlations among size-related morphological traits and the contents of lipids and proteins, documenting clade-wide trade-offs between morphological and nutritional fruit traits. Such negative covariances are expected to constrain the

evolutionary response of individual traits to selection imposed by seed dispersers and other agents. They were not detected for visual traits.

Biochemistry can at least partly explain the patterns of covariation among pulp nutrients e.g. the negative covariation between hydrophobic lipids and hydrophilic soluble carbohydrates. Similarly, negative covariation between soluble carbohydrates and the contents of protein and fibre were found previously (e.g. Herrera, 1987; Schaefer *et al.*, 2003b) and are possibly attributable to distinct biochemical pathways and metabolic constraints. Finally, among the colour components, hue and chroma (saturation) show similar covariation, which might be because of the fact that most fruits with high saturation corresponded to orange to red fruits with high hue values in our sample.

We found significant positive covariance between some nutritional and visual fruit traits throughout plant phylogeny. Again, consideration of biochemical pathways contributes to explaining this covariance. For example, brightness and carbohydrates probably covary because carbohydrates up-regulate the biosynthesis of the major fruit pigments, anthocyanins (Hu *et al.*, 2002; Solfanelli *et al.*, 2006). Also, sugars are normally present in the chemical structure of anthocyanins (van Buren, 1970). Similarly, the other major fruit pigments, carotenes, are always associated with lipid molecules because their biogenesis is dependent upon a phospholipid environment (e.g. Camara & Brangeon, 1981), which probably explains the marked covariation of lipids with hue and chroma throughout the phylogeny.

As nutritional and colour traits covary, frugivorous animals can use visual stimuli as indicators of nutritional rewards. Selecting colours as reliable indicators of nutritional rewards will – in addition to fruit biochemistry – enforce the covariance between both groups of traits. Because our analysis of PI documents that visual and nutritional fruit traits are less constrained than morphological ones, we suggest that correlated selection by frugivores may contribute to the evolutionary covariance among nutritional and visual fruit traits.

Analysing PI among fruit traits provides a strong test for the ‘seed dispersal syndrome concept’. This concept defines syndromes as sets of correlated phenotypic traits across distantly related taxa that are associated with particular guilds of frugivores (e.g. van der Pijl, 1972; Janson, 1983; Fisher & Chapman, 1993; Chen *et al.*, 2004; Lomáscolo *et al.*, 2008, 2010). While the concept of syndromes implicitly assumes PI among traits, it fails to specify the relative strength of the integration. Here, we show (i) that morphological traits are better predictors of syndromes than nutritional and colour traits (see Fig. 3), (ii) trade-offs between fruit size and the contents of lipids and protein, and (iii) that only a small set of fruit traits interacting with different sensory modalities (i.e. brightness and carbohydrates as well as hue, chroma and lipids) are correlated positively through phylogenetic

history. Thus, we show that only a limited set of fruit traits in the Mediterranean area exhibits the positive or negative covariation among morphological, nutritional and visual traits that the seed dispersal syndrome concept predicts.

### Fruit integration and frugivore types

We found that fruits consumed by birds showed relatively higher integration than fruits consumed by both birds and mammals or by mammals only. This distinct typology of fruits is likely to be explicable by differences in the foraging pattern, the digestive physiology and sensory ecology of birds and mammals. Based on the differences in PI values, we predict that a wider range of morphological, nutritional and visual fruit trait combinations can evolve in subclades of plants that are mainly dispersed by mammals compared to those with birds as their main seed dispersers.

The higher morphological integration of fruits consumed by birds is probably associated with stronger ecomorphological constraints acting on birds, where gape size sets a limit for the size and shape of fruits typically consumed by them. For example, fruit selection is a function of body (and gape) size in birds because handling constraints increase with increasing fruit size for gape-limited frugivores like birds (e.g. Wheelwright, 1985; Jordano, 1987, 1995b). Because mammals tend to be larger and span a larger range of body sizes, they are less likely to exert consistent selection upon fruit morphology. Our results suggest that the size of frugivore groups played a central role in the diversification of fleshy fruit sizes.

The higher integration values of nutrients in fruits used by birds relative to fruits that are solely or largely consumed by mammals are likely to be related to the fine-tuned discrimination abilities of birds for specific combinations of nutrients (Jordano, 1988). Consistent avian preferences for certain nutrient combinations (Schaefer *et al.*, 2003a) may produce consistent covariation among nutritional traits, thereby explaining the higher PI values of bird-dispersed fruits. By contrast, carnivorous mammals have more generalized diets, with large animal prey fractions in addition to fruits, and are presumably less selective in their fruit choice.

Finally, supporting the hypothesis that birds are more visually guided foragers than mammals, we found that the distinct components of colours of fruits consumed by birds (chroma, hue and brightness) are more integrated than the colour of fruits consumed largely or exclusively by mammals. This difference is likely explicable by the distinct visual systems of both groups. Birds use four different cone types to perceive colours, whereas frugivorous mammals use two different cone types. The higher number of cone types entails that diurnal birds can better discriminate fruit colours and their backgrounds than partly nocturnal mammals.

In summary, we demonstrate that birds and mammals can modulate the phenotypic space of fleshy fruit displays differently. It remains to be seen whether this result holds consistently across different floras where the sensory systems of both birds and mammals may differ from those in the Mediterranean area. Moreover, it would be interesting to analyse whether variation in PI that could be attributable to distinct frugivore groups has resulted in variable diversification patterns of fruiting displays among major angiosperm subclades. Finally, we propose that PI in fruits should be tested in microevolutionary studies both among and within populations. Studying PI thus allows testing whether adaptations to mutualistic partners or internal constraints favour certain combination of traits. In the case of plant–animal mutualisms that depend on highly diversified interactions, it is fundamental to consider that the potential selective pressures act on complex suites of displays rather than on single phenotypic traits.

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### Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Phylogenetic arrangement of species in the data set used for comparative analyses (see also Appendix S2 for literature sources).

**Table S1** Dataset of fruit variables (morphology, pulp nutrient, and colour) (see also Appendix S1 for literature sources).

**Table S2** Correlation matrix of phylogenetically independent contrasts (PIC).

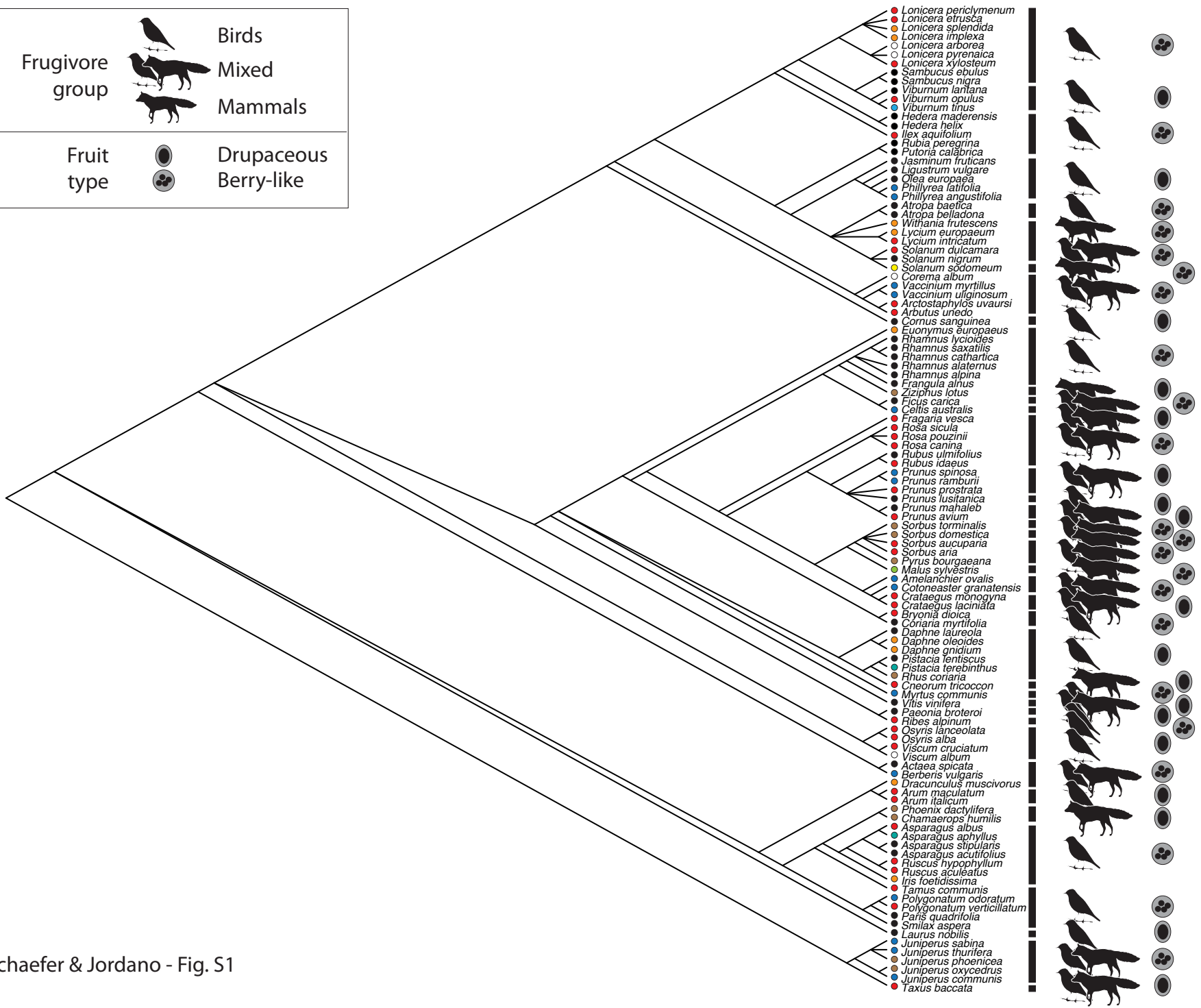
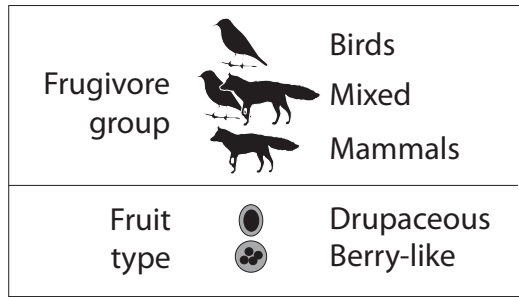
**Appendix S1** List of literature sources for Table S1.

**Appendix S2** List of literature sources for Figure S1.

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Valido, Schaefer & Jordano - Fig. S1

**Table S2.** Correlation matrix of phylogenetically-independent contrasts (PIC). 13x13. Significant correlations are in bold and significance values are as follows: \*\*\*, P< 0.001; \*\*, P< 0.01; \*, P< 0.05. Marginally significant correlations (0.05 < P < 0.10) are underlined.

	diam	frfm	pdm	sdm	seeds	lip	pro	nsc	ash	fib_ac	bright	hue	chrom
diam	-												
frfm	<b>0.961***</b>	-											
pdm	<b>0.861***</b>	<b>0.874***</b>	-										
sdm	<b>0.606***</b>	<b>0.626***</b>	<b>0.598***</b>	-									
seeds	<b>0.384***</b>	<b>0.296**</b>	<u>0.175</u>	<u>0.187</u>	-								
lip	<b>-0.802**</b>	<b>-0.275**</b>	<b>-0.160*</b>	-0.066	-0.304	-							
pro	<b>-0.614*</b>	<b>-0.070*</b>	<b>-0.060*</b>	-0.004	0.126	<u>0.245</u>	-						
nsc	0.163	0.0211	0.009	-0.006	-0.022	<b>-0.663***</b>	<b>-0.483***</b>	-					
ash	-0.332	-0.058	<b>-0.068*</b>	0.017	<u>0.193</u>	-0.147	<b>0.282**</b>	<b>0.432**</b>	-				
fib_ac	0.491	0.053	0.038	0.033	-0.040	<b>0.254*</b>	0.116	<b>-0.330***</b>	-0.127	-			
bright	0.067	0.108	<u>0.135</u>	0.015	-0.069	-0.479	<b>-0.686**</b>	<u>0.498</u>	<b>-0.894**</b>	0.240	-		
hue	-0.066	-0.084	<u>-0.131</u>	-0.054	-0.025	<u>0.662</u>	-0.425	0.373	0.131	<b>-0.589*</b>	0.113	-	
chrom	0.041	0.039	-0.012	0.018	0.026	-0.032	-0.052	0.005	0.057	-0.037	<b>0.400***</b>	<b>0.298**</b>	-



## Supplementary Material

### Appendix S1: List of references and databases used for obtaining frugivorous interactions for the Mediterranean plant species (ref\_frug), and information about morphology (ref\_morph) and pulp nutrients (ref\_nutr) of the fleshy fruits cited in the Fruit\_Database (Table S1).

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## Supplementary Material

### Appendix S2: List of references and databases used for obtaining plant phylogeny used in Figure S1.

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