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Correlated evolution of fig size and color supports the dispersal syndromes hypothesis

**Silvia B. Lomáscolo · Pablo Speranza ·
Rebecca T. Kimball**

Abstract The influence of seed dispersers on the evolution of fruit traits remains controversial, largely because most studies have failed to account for phylogeny and or have focused on conservative taxonomic levels. Under the hypothesis that fruit traits have evolved in response to different sets of selective pressures by disparate types of seed dispersers (the dispersal syndromes hypothesis), we test for two dispersal syndromes, defined as groups of fruit traits that appear together more often than expected by chance. (1) Bird syndrome fruits are brightly colored and small, because birds have acute color vision, and commonly swallow fruits whole. (2) Mammal syndrome fruits are dull-colored and larger on average than bird syndrome fruits, because mammals do not rely heavily on visual cues for finding fruits, and can eat fruits piecemeal. If, instead, phylogenetic inertia determines the co-occurrence of fruit size and color, we will observe that specific combinations of size and color evolved in a small number of ancestral species. We performed a comparative analysis of fruit traits for 64 species of *Ficus* (Moraceae), based on a phylogeny we constructed using nuclear ribosomal DNA. Using a concentrated changes test and assuming fruit color is an independent variable, we found that small-sized fruits evolve on branches with red and purple figs, as predicted by the dispersal syndromes hypothesis. When using diameter as the independent variable, results vary with the combination of algorithms used, which is discussed in detail. A likelihood ratio test confirms the pattern found with the concentrated changes test using color as the independent variable. These results support the dispersal syndromes hypothesis.

Keywords Concentrated changes test · *Ficus* ·
Fruit evolution · Likelihood ratio test · Seed dispersal

Introduction

Although seed dispersers clearly influence the fitness and spatial distribution of fruiting plants (Harms et al. 2000; Howe and Miriti 2000; Márquez et al. 2004; Terborgh et al. 2002), their effect on the evolution of fruit traits remains controversial (Bolmgren and Eriksson 2005; Fischer and Chapman 1993; Herrera 1992; Jordano 1995; Lord et al. 2002; Mack 1993). Evolutionary ecologists have proposed that differences in the behavior and ecology of seed dispersers have led to corresponding differences in fruit species favored by those dispersers (Gautier-Hion et al. 1985; Janson 1983; Kalko et al. 1996, Schaefer et al. 2006). For example, fruit-eating birds are diurnal, have excellent color vision, a poor sense of smell, and are limited in terms of the size of fruit they can eat by gape width (Jordano 2000; Levey 1987; Rey et al. 1997; Wheelwright and Janson 1985). Therefore, fruiting plants dispersed by birds should tend to stand out against the green foliage, and be odorless and small. Colors that stand out against leaves include red, black, and purple, all of which have either high chromatic or achromatic contrast against foliage (Burns and Dalen 2002; Janson 1983; Schmidt et al. 2004). Fruit-

S. B. Lomáscolo (✉) · R. T. Kimball
Department of Zoology, University of Florida,
P.O. Box 118525, Gainesville, FL, USA
e-mail: slomascolo@zoo.ufl.edu

P. Speranza
Department of Plant Biology, Facultad de Agronomía,
Universidad de la República, Av. E. Garzón 780,
Montevideo 12900, Uruguay

eating mammals, on the other hand, are often nocturnal, have teeth (i.e., are not gape-limited), and exhibit a keen sense of smell. Thus, mammal-dispersed fruits do not need to stand out against green foliage (e.g., green, yellow, brown, or pale orange; Janson 1983), and are more likely to be relatively large and odoriferous.

These sets of fruit traits define what have been termed "bird syndrome" and "mammal syndrome" fruits, respectively (Herrera 2002; McKey 1975; van der Pijl 1969). At least superficially, fruit traits such as size and color seem to be associated nonrandomly, as expected (Gautier-Hion et al. 1985; Janson 1983; Kalko et al. 1996; Korine et al. 2000; Shanahan and Compton 2001; Wheelwright and Janson 1985). However, two opposing hypotheses explain the co-occurrence of fruit size and color. A non-adaptive hypothesis is that co-occurrence of fruit traits is phylogenetically determined; the size and color of a given fruit species is most strongly determined by the size and color of ancestral species (the phylogenetic inertia hypothesis). Under this hypothesis, frugivores have pre-existing preferences for particular types of fruits and disperse seeds in fruits that match those preferences, an apparently common process called "ecological fitting" (Agosta 2006; Janzen 1980; Janzen 1985; Strauss et al. 2006) that does not drive the evolution of fruit traits. Alternatively, the co-occurrence of fruit traits is determined by selective pressure from sets of seed dispersers that differ in behavior and ecology, as described above (dispersal syndromes hypothesis).

Tests of these hypotheses with phylogenetic comparative methods have commonly rejected the dispersal syndromes hypothesis (Fischer and Chapman 1993; Herrera 1987, 1989, 1992; but see Jordano 1995). However, this result may reflect methodological challenges more than biology. In particular, these studies have pooled data from species of the same genus (Fischer and Chapman 1993), or they have focused on temperate species (Herrera 1987, 1989, 1992), thereby overlooking immense intrageneric variation, especially in tropical taxa. This variation remains largely unexplored in phylogenetic comparative analyses.

Several lines of evidence from studies with a broad geographic scope support the dispersal syndromes hypothesis. These studies controlled for phylogenetic inertia by comparing fruit traits among plants of the same genus (Lord 2004; Lord et al. 2002), family (Mack 1993) or species (Hampe and Bairlein 2000). For example, fruit traits in New Zealand are consistent with hypothesized selective pressures by avian and reptilian frugivores, the only frugivores historically present in New Zealand (Lord 2004; Lord et al. 2002). Also, tropical fruits in the New World tend to be smaller than related plant species in the Old World (Mack 1993), which is consistent with the observation that New World mammalian frugivores are

smaller than in the Old World (Fleming et al. 1987; Mack 1993). Lastly, within-species differences in fruit traits match the characteristics of the different disperser guilds found in two different regions of Europe (Hampe and Bairlein 2000). Despite these seemingly non-random patterns, such studies are limited by low replication (typically, one region is compared to another) and lack of an explicit phylogenetic framework.

We provide a phylogenetic test of the dispersal syndromes hypothesis at the species level in the genus *Ficus* (Moraceae) by testing two predictions that arise under this hypothesis: (1) *Ficus* species with ripe fruit that are red or purple will produce smaller fruit than species with green, yellow, brown, and orange fruits, and these combinations of color and size will occur independently in different clades; and (2) interspecific variation in fruit size of red, purple, or black figs will be less than the interspecific variation in fruit size of green, yellow, brown, or orange figs. The rationale for the second prediction is that birds are more restricted than mammals in the size of fruits they can ingest, because mammals can manipulate fruits with their forelimbs and can eat them piecemeal using their teeth (Alcantara and Rey 2003). Although disperser guilds such as birds and mammals include animals that vary considerably within each group (e.g., tanagers to cassowaries, and bats to elephants) (Lord et al. 2002), we are testing traits that should be affected in a similar way by most members of each guild. We believe it is a fair generalization to say that most birds have more acute color vision than most mammals (especially nocturnal mammals) (Bowmaker et al. 1997; Bowmaker and Hunt 2006; Das et al. 1999), and that, overall, mammals are larger than birds and can therefore feed on larger fruits than most birds. Even smaller mammals, such as bats, rodents, and many marsupials, may be able to eat larger fruits than birds of comparable size because they have teeth and can eat the fruits piecemeal, and they have forelimbs and can handle the fruits in more ways than birds can with their beaks. Through this conservative test of the dispersal syndromes hypothesis, we found that color and size of figs seem to evolve in correlation. The patterns of correlated evolution were identified *despite* the conservative nature of our test, given such broad disperser guilds.

Methods

Study system

Ficus is an ideal group for testing the dispersal syndromes hypothesis because its fruits vary immensely among its ca. 750 species. Figs can be yellow, brown, red, purple, or

orange, and may be as small as 4 mm in diameter to as big as 80 mm (Dominy et al. 2003; Laman and Weiblen 1998; S. Lomáscolo, personal observation). These species have an unusually wide distribution, as they are found in almost all tropical forests worldwide, and their fruits are consumed by a taxonomically diverse array of vertebrates, including 1,274 bird and mammal species in 523 genera, in addition to reptiles and fishes (Shanahan et al. 2001). Furthermore, molecular data are available to build a phylogeny and fruit size and color are described in the literature for many species. We used 64 *Ficus* species from throughout its range, for which genetic and fruit size and color information were available in the literature.

General approach

We first built a molecular phylogeny of the genus *Ficus*, which is necessary to tease apart the dispersal syndromes hypothesis from the phylogenetic inertia hypothesis. To ensure that the reconstructed phylogeny was not affected by the method chosen to build it, we constructed phylogenies using maximum likelihood (ML), Bayesian, and parsimony searches.

Correlated evolution of fruit color and size was tested using Maddison's (1990) concentrated changes test and Pagel's (1994) Likelihood Ratio test on the parsimony and ML trees.

Because phylogenetic comparative methods give priority to phylogeny over ecology when explaining shared characters (Westoby et al. 1995), and we do not know the degree of phylogenetic niche conservatism (Harvey and Pagel 1991) in figs, we also tested for correlation of fruit color and size using raw data (hereafter *nonphylogenetic* comparative analyses). Nonphylogenetic comparative analyses were performed on the set of species that is included in our phylogeny and on all *Ficus* species for which fruit data were available in the literature. This allowed us to test whether the limited set of species in the phylogeny was representative of a much larger set of species. The second prediction was tested without incorporating phylogeny, because all phylogenetic comparative tests that we are aware of require data for each species in the phylogeny to reconstruct ancestral character state. Because we are testing *variation* in fruit size between the two types of fruits, to do a phylogenetic analysis we would need data on *intra*specific fruit size variation for each species in our phylogeny, which was not available in the literature. In most cases, only a mean diameter was reported without any measure of within-species variation. Thus, we calculated *inter*specific variation for mammal-colored and bird-colored fruits, and compared them using an *F* test. This approach assumes that selective pressure on fruit size by birds and mammals operates on figs at the community level.

Fruit trait data

Fruit trait data were obtained primarily from published sources (Croat 1978; Dominy et al. 2003; Laman and Weiblen 1998), and secondarily from a *Ficus* expert (Weiblen, personal communication) and field data collected by S. Lomáscolo. All data are from ripe fruits; unripe fruits were excluded. Because the phylogenetic comparative tests we used are designed for dichotomous variables, we transformed color data into two categories, following Janson's (1983) classification of Type A (red and purple) and Type B (green, yellow, brown, and orange) fruits. Likewise, size data were transformed into two categories, based on the mode diameter (10 mm) of species included in our study: "small" ($\leq$10 mm) and "large" (>10 mm). The 10 mm cutoff point also coincides with the mode for all *Ficus* species for which diameter data were available in the literature (Croat 1978; Dominy et al. 2003; Weiblen, personal communication; Laman and Weiblen 1998), with the mean fruit diameter and bird gape width reported by Wheelwright (1985), and with the largest diameter of fruits typically consumed by some of the most common passerine birds in Costa Rica (Levey 1987).

Occasionally, different sources provided contradictory information about the fruit color of a given species. For example, *Ficus asperifolia* is reported to produce yellow or red fruits (Dominy et al. 2003), which means that it could be classified as either Type A or B. Because we cannot discern whether these situations result from a polymorphism, phenotypic plasticity, or simply a reporting error, we performed separate analyses for each possible assignment of color type. We reconstructed character change in two ways. For the most conservative reconstruction, we chose the option that would minimize support for the dispersal syndromes hypothesis. For example, if a species had either yellow or red figs and was classified in the "small" category, we recorded it as Type B, so that the combination of color and size would not match the prediction of Type A color and small size. The second reconstruction was the nonconservative reconstruction where, for each ambiguous classification, we chose the color that matched the hypothesis of correlated evolution between the two traits. By comparing the results of these two approaches, we are able to assess the sensitivity of studies like ours to sometimes arbitrary character state reconstruction—a frequent limitation of data gleaned from the literature.

Phylogenetic data and analyses

Although three phylogenies have been published for *Ficus*, the comparative tests that we used require a phylogeny that has branch lengths and includes only the species for which fruit data were available (likelihood ratio test, Pagel 1994),

or else a phylogeny that is completely resolved (concentrated changes test, Maddison 1990). Thus, we constructed our own phylogeny.

Sequences

Internal transcribed spacer (ITS) sequences for 58 *Ficus* species were obtained from GenBank. For six additional species (*F. congensis*, *F. exasperata*, *F. mucuso*, *F. natalensis*, *F. asperifolia 2*, *F. vallis-choudae*), we sequenced the ITS region. Voucher specimens for these species were deposited at the Florida Museum of Natural History Herbarium under numbers FLAS 218158–FLAS 218164, and the resulting ITS sequences were submitted to Genbank (Accession nos. EF520706–EF520713). *Ficus* ITS1 through ITS2 sequences were amplified using primers ITS1 through 5 (White et al. 1990). All PCR amplifications were carried out in 25 μL reactions containing 0.4 U of NEB Taq polymerase (New England Biolabs, Beverly, MA, USA), 1.5 mM $MgCl_2$, 0.4 μM of each primer and 0.1 mM of each dNTP in the manufacturer's buffer. Amplification was carried out in a Biometra® T3 Thermoblock with the same program for all primer combinations. The program consisted of an initial denaturing step of 95°C for 2 min; five cycles of 95°C for 1 min, 53°C for 1 min, and 72°C for 2 min, with a decrease of 1°C per cycle in the annealing temperature; 35 cycles with an annealing temperature of 48°C, and a final extension step of 72°C for 12 min. PCR products were cleaned with Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) and diluted to approximately 1 ng/μL for sequencing.

We sequenced all regions in both directions on a CEQ 8000 capillary sequencer (Beckman-Coulter, Fullerton, CA, USA) using one-quarter reaction volumes with the addition of 80 mM Tris and 2 mM $MgCl_2$ (pH 9) to complete the volume of a full reaction. To edit the sequences manually, we used Sequencher™ (V4.1.4, Genecodes, Ann Arbor, MI, USA).

Alignment

We prealigned the sequences with the Clustal-W (Thompson et al. 1994) algorithm included in BioEdit (V 5.0.6, Hall 1997) and manually adjusted the resulting alignment. The nrDNA region includes ITS1 and ITS2, separated by the 5.8S ribosomal RNA gene. Because the 5.8S region differed from ITS1 and 2 in that it contained few variable sites and appeared to evolve under a different model of evolution (see below), the 5.8S region was excluded from analyses. Base positions that could not be unambiguously aligned, particularly due to insertions or deletions (indels) of nucleotides, were recoded as missing data. There were 15 indels two or more bp in length in

regions where alignment was unambiguous. For parsimony analyses only, these were considered single characters, coded as present/absent, and included in analyses. Otherwise, all single bp deletions and multiple bp deletions that could not be unambiguously aligned were treated as missing data. A low-complexity region ranging from 20 to 40 bp in ITS 2 containing a poly-G was not readily alignable; however, preliminary parsimony searches conducted without this region did not result in sufficiently resolved trees. To make use of the information contained in this region, clades that had appeared consistently in our preliminary analyses and were congruent with the phylogenies shown in Weiblen (2000), Jousselin et al. (2003), and Rønsted et al. (2005) were aligned separately for this region. This approach is similar to the stepwise alignment strategy described by Hsiao et al. (1999). Although this strategy resulted in unambiguous within-clade alignments, alignment across clades remained difficult for this region. Consequently, in order to avoid spuriously reinforcing support for the clades that had been aligned separately, all informative positions within this region were manually de-aligned between pre-aligned groups by introducing gaps when the individual alignments were pooled in a single matrix.

Phylogenetic analyses

We used the clade formed by *F. maxima*, *F. insipida* and *F. yoponensis* as a functional outgroup based on the fact that it appeared as sister to the rest of the genus in all our preliminary analyses conducted using *Morus alba* as an outgroup. This topology was fully consistent with the analyses of Weiblen (2000), Jousselin et al. (2003), and Rønsted et al. (2005), performed with different data sets.

The maximum parsimony criterion searches for the phylogenetic tree that minimizes the number of evolutionary changes that must have occurred. Using PAUP* 4.0b10 (Swofford 2003), a preliminary heuristic search was performed with tree bisection-reconnection (TBR) branch swapping and the minimum tree length recorded. The final searches were conducted for 5,000 sequence addition replicates (SAR) by saving only trees that were shorter or equal to the minimum tree found in the preliminary search. A bootstrap analysis was also conducted for 1,000 bootstrap replicates with 100 SAR within each replicate using TBR.

The ML criterion searches for the phylogenetic tree that best fits the data, given a particular model of evolution. The model of evolution that best describes our data was determined by the hierarchical likelihood ratio test in MODELTEST 3.06 (Posada and Crandall 1998). We initially examined the best model for ITS1, ITS2, and the 5.8S regions independently. The best models selected for ITS1 and ITS2 were similar and more complex than the model

selected for the 5.8S region. Thus, the 5.8S region was excluded from analyses and the best model for the combined ITS regions was used for the likelihood analysis. We conducted a heuristic search in PAUP* with ten random sequence additions using the model (TN93) and parameters recommended by MODELTEST 3.06 (Posada and Crandall 1998).

We assessed support on the phylogeny using two different approaches. Bayesian analyses were conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). We ran our chains for 1×10^7 generations, sampling every 1,000 generations. The first 2×10^6 generations were discarded as "burn-in," and a consensus of the remaining trees was used to determine the posterior probabilities at each node. We also ran 200 bootstrap replicates using ML using GARLI 0.951 (Zwickl 2006). We considered nodes strongly supported when posterior probabilities were greater than 95% or when bootstrap values were greater than 70% (Alfaro et al. 2003; Hillis and Bull 1993).

Fruit trait analyses

Phylogenetic comparative analysis

To test whether the pattern of association between fruit color and size constitutes evidence of correlated evolutionary change in both traits (prediction #1), we performed Pagel's Likelihood Ratio test (1994), as implemented in Discrete v.4.0 (Pagel 1994, 1999), which compares the goodness of fit of a model of independent evolution to one of correlated evolution. This test assumes that changes in character state are more probable on long branches than on short branches, and takes into account branch length for character state reconstruction. We also performed Maddison's (1990) concentrated changes test using MacClade 4.0 (Maddison and Maddison 2000), which tests for nonrandom association of traits by determining if an evolutionary change in one trait is correlated with change in a second trait. The test reconstructs character changes using a parsimony criterion and assumes a dependent and an independent variable. We determined whether color or size was more likely to be independent using Discrete (Pagel 1994, 1997). For example, if changes in color precede changes in size, rates of change in size should differ depending upon color type, while rates of color change should not differ by size category. By constraining rates of change among joint character states (e.g., small Type A to small Type B set equal to large Type A to large Type B), the likelihood of color preceding size could be compared to that of size preceding color. Since this approach does not test nested models, the different models were compared using AIC (Akaike's information criterion) (Burnham and

Anderson 2003). Since the results of this test were not conclusive, and since the mode of evolution of fruit traits is not well understood, we performed the concentrated changes test Maddison (1990) in two different ways: first using color as the independent variable, and then using size as the independent variable.

The concentrated changes test cannot deal with unresolved nodes, so we resolved the polytomies in our phylogeny by using the resolution of either our majority rule tree or the reconstructions of Weiblen (2000), Jousselin et al. (2003), or Rønsted et al. (2005).

Sensitivity analysis

The concentrated changes test requires the specification of several different algorithms, although algorithm definition may be arbitrary if the mode of trait evolution is not well known. To address this, we performed a sensitivity analysis using all available algorithm combinations to explore whether algorithm choice had a major influence on the test of prediction #1 and, if so, which algorithm seemed to most influence the results. The algorithms that we varied were (1) ACCTRAN or DELTRAN, which are options for resolving ambiguous branches and that produce character changes earlier (at the basal branches) or later (at the terminal branches) in phylogenetic history, respectively. ACCTRAN increases the number of losses, whereas DELTRAN increases the number of gains; and (2) MINSTATE or MAXSTATE, which are options for reconstructing character states at ambiguous nodes. MINSTATE uses the lowest trait value, whereas MAXSTATE uses the largest trait value.

As part of the sensitivity analysis, we ran the analyses using conservative and nonconservative trait reconstruction, using color and size as the independent variables.

Nonphylogenetic comparative analysis

To test if Type A fruits had smaller mean diameter than Type B fruits (prediction #1), we used a Kolmogorov–Smirnov nonparametric test because our data were not normal. To test whether Type B fruits have greater variation in fruit size than Type A fruits (prediction #2), we performed an F test (Zar 1999). Both tests were performed for all species of *Ficus* found in the literature and for the subset of species included in the phylogeny.

Results

Phylogenetic reconstruction

The phylogenies generated using ML, Bayesian, and parsimony analyses were similar to each other, as well as to

published phylogenies (Jousselin et al. 2003; Rønsted et al. 2005; Weiblen 2000). The majority of nodes were well-supported in our analyses (Fig. 1). With taxa shared among our phylogenies and published phylogenies, all of the major clades with high bootstrap values were essentially identical. Minor conflicts occurred only in poorly supported groups. Slight differences in topology between the ML and parsimony tree did not affect the results of our phylogenetic comparative analyses (see below).

Nonphylogenetic comparative analysis

When we analyzed data only for those species of *Ficus* included in our phylogeny, we omitted a species (*F. ruginerva*) whose fruits were reported as being larger than 10 mm, but no exact diameter was given. For this limited set of species, Type A fruits were smaller than Type B fruits, in agreement with prediction #1 ($\bar{x}_A = 14.86$ mm, $n_A = 36$; $\bar{x}_B = 27.41$, $n_B = 27$; Kolmogorov–Smirnov $Z = 1.818$; P value $= 0.003$). For the entire set of species, the difference was in the same direction but not as significant ($\bar{x}_A = 15.46$ mm, $n_A = 56$; $\bar{x}_B = 18.13$ mm, $n_B = 72$; Kolmogorov–Smirnov $Z = 1.359$; P value $= 0.050$). The variance in the diameter of Type B fruits was significantly larger than the variance of Type A fruits for the subset of species in our phylogeny, in agreement with prediction #2 ($\sigma_A^2 = 79.63$; $\sigma_B^2 = 281.69$; $F = 3.54$; df numerator $= 35$, df denominator $= 26$; P value < 0.001). However, this difference in variance was not significant when all species were included in the analysis ($\sigma_A^2 = 106.68$; $\sigma_B^2 = 116.36$; $F = 1.09$; df numerator $= 71$, df denominator $= 55$; one-tailed test P value > 0.25) (Fig. 2).

Phylogenetic comparative analysis

For two reasons, we emphasize the analyses performed on the ML tree (Fig. 3). First, Pagel's Likelihood Ratio test (1994) analysis requires branch lengths, which were only available on the ML trees. Second, the parsimony searches came up with over 5,000 most parsimonious trees and so it was unwieldy to perform the comparative analyses in all 5,000 of them. A preliminary comparative analysis performed on a randomly chosen parsimony tree showed no difference from the comparative analyses performed on the ML tree.
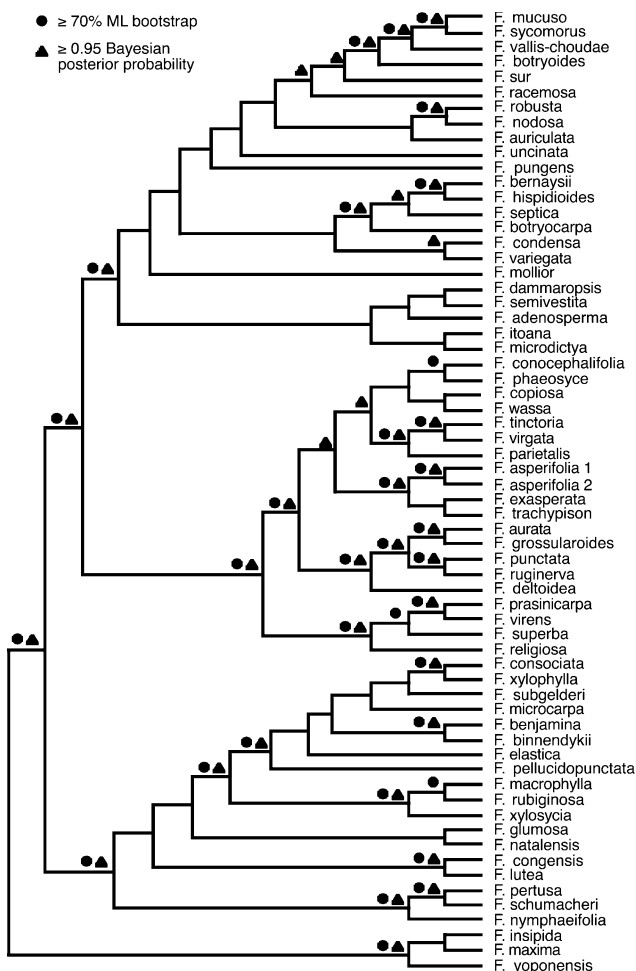

Fig. 1 Bootstrap support and Bayesian posterior probabilities on the maximum likelihood (*ML*) phylogeny of 64 *Ficus* species used for analyses. Nodes with strong bootstrap support in ML analyses (≥70%) are indicated with a *circle*; nodes with strong support using Bayesian posterior probabilities (≥0.95) are indicated with a *triangle*
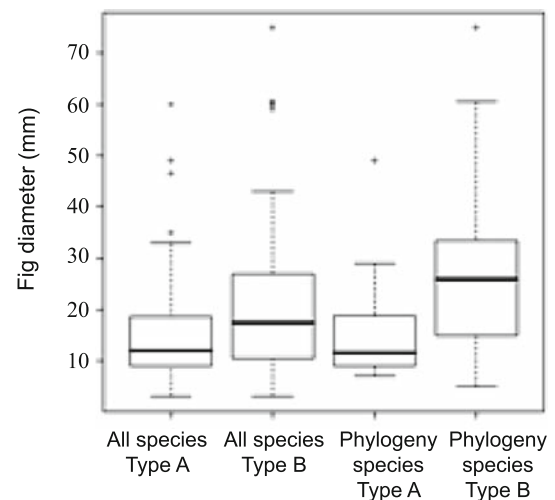

Fig. 2 Box plot comparing the mean diameters of Type A (red, purple) and Type B (green, yellow, brown, and orange) fruits (Janson 1983) for the set of *Ficus* species in our phylogeny ($n_A = 36$, $n_B = 27$) and for all *Ficus* species found in the literature ($n_A = 56$, $n_B = 72$). The *box* indicates the first and third quartiles and the *horizontal line* inside the boxes marks the median. The *vertical lines* show the largest and smallest observations that fall within 1.5 times the box size from the nearest quartile
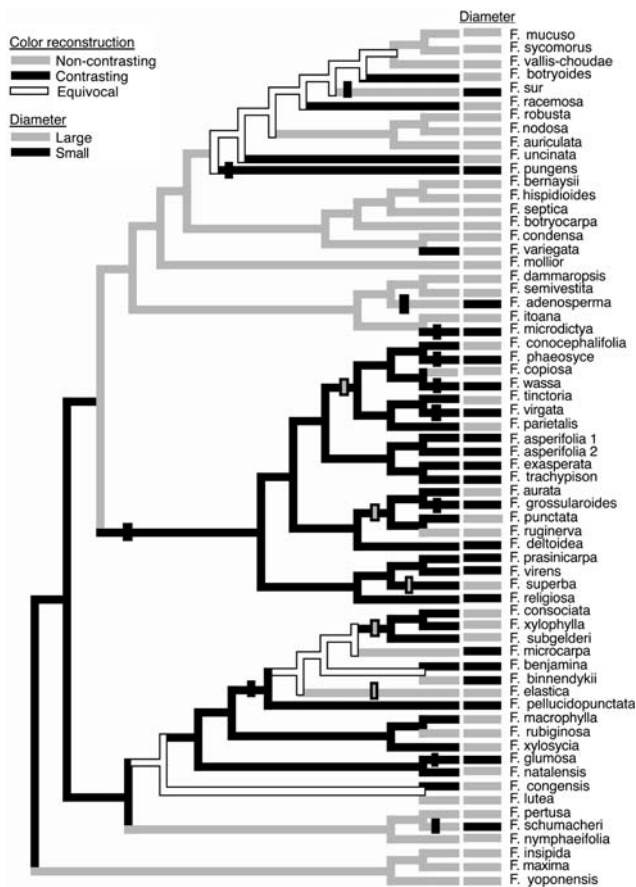
Fig. 3 Maximum likelihood phylogeny of 64 *Ficus* species. Color is mapped onto the phylogeny and was done using a parsimony criterion in MacClade (the only criterion available in this software), and is one of the reconstructions tested with the concentrated changes test. *Black branches* represent Type A colored fruits, *gray branches* represent Type B colored fruits, and *white branches* represent ambiguous reconstruction. At the tips of the branches, character state for diameter is mapped for each fig species. Forty-two species show the combinations predicted by the dispersal syndrome hypothesis (Type A colors and small size, or Type B colors and large size), whereas 22 species show a mismatching combination. The ancestral state for fruit diameter was reconstructed as large. Changes of diameter from large to small (called gains, and represented by *white dashes*) and small to large (called losses, and represented by *gray dashes*) are marked on the branches, where they are hypothesized to have occurred according to the parsimony criterion used for the reconstruction. For this particular reconstruction, out of a total of 12 gains and 5 losses of small size, 8 and 4, respectively, occurred on branches with Type A colored fruits. For branch support value using ML bootstrap and Bayesian posterior probability, see Fig. 1

With Pagel's likelihood ratio test (Pagel 1994), the model of correlated evolution of fruit size and color fits the data significantly better than the model of independent evolution, regardless of the coding of ambiguous fruit colors (conservative reconstruction: likelihood ratio = 4.889; *P* value = 0.027; 1,000 simulations. Nonconservative reconstruction: likelihood ratio = 4.990; *P* value = 0.027; 1,026 simulations).

To determine the independent variable for Maddison's concentrated changes test (Maddison 1990), we calculated the likelihood of color preceding size (ln likelihood = −80.31) and that of size preceding color (ln likelihood = −81.68). However, while color may be more likely to precede size, examination of the Akaike weights cannot exclude size preceding color. Assuming color is independent is more consistent with the observation that color tends to change at basal branches while size tends to change at more terminal branches (Fig. 3). When color is considered the independent variable, the results for Maddison's concentrated changes test (Maddison 1990) indicate that fruit size changes from large to small on branches with brightly colored fruits more often than expected by chance. The *P* values from all the tests performed with the different algorithm combinations and trait reconstructions ranged from 0.002 to 0.03 (Table 1). When size is considered the independent variable, however, results vary greatly according to the algorithm combination and character state reconstruction used ($0.04 < P$ value < 0.54; Table 1).

Discussion

Overall, our results support the dispersal syndromes hypothesis. Mean diameter is smaller for Type A fruits than for Type B fruits, supporting prediction #1 of the dispersal syndromes hypothesis. This pattern holds when using raw species data for the subset of species included in our phylogeny and using raw data for all of the species of figs found in the literature. It also holds when examined with phylogenetic comparative methods: the size of fruits tends to change from large to small on species with Type A colors (e.g., red and purple) more often than expected by chance. This result was consistent across all combinations of algorithms for the concentrated changes test (Maddison 1990) when color was considered the independent variable. The tendency of color and size to evolve in correlation, at least in the subset of 64 species of *Ficus* included in our analyses, supports the dispersal syndromes hypothesis rather than the phylogenetic inertia hypothesis, suggesting that seed dispersers have been important for the evolution of at least some fruit traits in *Ficus*.

The dispersal syndromes hypothesis was supported to a lesser extent by our test of prediction #2. In the subset of species used in our phylogeny, Type A fruits are significantly less variable among species in diameter than Type B fruits, as predicted. However, in analyzing the variation of this type, we could not control for phylogeny and the significance of the difference in variation between Type A and B fruits disappeared when all species were included.

Table 1 Results from Maddison's concentrated changes tests showing all combinations of algorithms and character state reconstructions performed on the maximum likelihood tree using color and size as the independent variable (I and II, respectively)

	Independent variable	Nonconservative reconstruction				Conservative reconstruction			
		Deltran		Acctran		Deltran		Acctran	
Resolving option to count gains and losses	Color	Total: 17 gains, 0 losses		Total: 12 gains, 5 losses		Total: 17 gains, 0 losses		Total: 12 gains, 5 losses	
		Branches: 12 gains, 0 losses		Branches: 9 gains, 4 losses		Branches:13 gains, 0 losses		Branches: 9 gains, 4 losses	
		minstate	maxstate	minstate	maxstate	minstate	maxstate	minstate	maxstate
P value		0.012	0.005	0.011	0.007	0.002	0.002	0.034	0.015
Resolving option to count gains and losses	Diameter	Total: 12 gains, 2 losses		Total: 7 gains, 7 losses		Total: 9 gains, 7 losses		Total: 9 gains, 7 losses	
		Branches: 5 gains, 0 losses		Branches: 3 gains, 1 loss		Branches: 2 gains, 3 losses		Branches: 3 gains, 2 losses	
		minstate	maxstate	minstate	maxstate	minstate	maxstate	minstate	maxstate
P value		0.131	0.085	0.049	0.067	0.539	0.544	0.197	0.204

Total, total number of gains and losses using each resolving option (DELTRAN or ACCTRAN). *Branches*, number of gains and losses on the branches with the character state of interest. When color is considered the independent variable, *Branches* is the total gains and losses of "small size" that occur on branches with Type A (brightly colored) fruits. When size is considered the independent variable, *Branches* is the total gains and losses of Type A colors on branches with small-sized fruits

Returning to prediction #1, an alternative hypothesis for the observed nonrandom combinations of fig color and size is physiological. Because the production of reproductive parts, including fruits, can be costly to plants (Jurik 1983; Reekie and Bazzaz 1987), and because green fruits can photosynthesize even after they are ripe, green fruits may have lower costs of production than fruits of other colors (Cipollini and Levey 1991). Given that large fruits must be more costly to produce than small fruits, green photosynthetic fruits should be favored over fruits of different colors. However, a positive carbon balance in green fruits has been found only at high light levels, and the balance becomes negative at low levels (Cipollini and Levey 1991). Because light levels may change considerably during a plant's lifetime, it seems unlikely that large fruits would be selectively advantageous as extra photosynthetic tissue, unless the plant grew exclusively in brightly lit habitats and ripe fruits were fully exposed to the sun. Also, considering the large surface area provided by leaves, it seems unlikely that photosynthetic ripe fruits would benefit the parent plant sufficiently to increase its fitness relative to other plants with fruits of a different color and a similar size.

The sensitivity analysis for Maddison's concentrated changes test (Maddison 1990) helped us to identify which source of variation has the stronger impact on our results, given the uncertainty in both the phylogenetic reconstruction and the mode of fruit trait evolution. The largest impact on the results was generated by switching color and size as independent variables in the concentrated changes test (Maddison 1990, Table 1). When using size as the independent variable, the tests' *P* value varied widely, especially between the conservative and nonconservative reconstructions. We think that the choice of the independent variable affects the results in our study because when size is the independent variable, few changes of color occur on branches with small-sized fruits, primarily since small-sized fruits appear in more terminal branches. When so few changes occur (a small sample size for the purpose of the concentrated changes test), calculating the probability of the observed number of gains and losses seems to be very sensitive to small changes in the number of gains and losses. This sensitivity highlights the importance of understanding the mode of fruit trait evolution (i.e., which trait evolves first) before one can draw conclusions about the importance of seed dispersers for the evolution of fruit traits. Even though the test used to decide which variable should be used as independent was fairly inconclusive, observing the pattern of gains and losses as reconstructed using a parsimony criterion gives us confidence that color can be considered the independent variable.

An important caveat of our methodology is the use of colors as perceived by humans. Many species of birds, for example, can detect UV (Bennett et al. 1994; Bennett and Thery 2007; Bowmaker et al. 1997; Das et al. 1999; Hart 2001; Schaefer et al. 2006). Likewise, cone type and color perception can vary widely in mammals (Bowmaker and Hunt 2006; Peichl 2005). Unfortunately, data that incorporate the whole range of wavelengths reflected by fruits, and color perception by different frugivores (Schaefer et al. 2006), are not available in the literature for the fig species

Table 2 Information on fruit diameter, color, animal consumers, and distribution for the 64 species included in our phylogeny, as found in the literature

Ficus species	Diameter (mm)	Color	Size code	Fruit type	Avian consumers[h]	Mammalian consumers[h]	Other consumers[h]	Distribution
F. adenosperma	10[a]	Green[a]	Small	B	No data found	No data found		New Guinea[b]
F. asperifolia	10[a]	Yellow or red[a]	Small	A or B	Pigeons	OW primates	Fish	Africa[a]
F. aurata	11[a]	Orange[a]	Large	A	Passeriforms	Rodents		Borneo[a]
F. auriculata	60[g]	Green[g]	Large	B		OW bats		China[b]
F. benjamina	8[a]	Purple[a]	Small	A	Galliforms, pisciforms, hornbills, cuculiforms, parrots, pigeons, passeriforms	NW and OW bats, OW primates, civet, rodents		Borneo[a]
F. bernaysii	12[f]	Red[f]	Large	B	Cassowary, hornbills parrots, pigeon, passeriforms	Marsupials, OW bats		New Guinea[b]
F. binnendykii	8[a]	Red[a]	Small	B	Pisciforms, hornbills, pigeons, passeriforms	OW primates, rodents		Peninsular Malaysia[a]
F. botryocarpa	35[a]	Green[a]	Large	B		Marsupials		New Guinea[b]
F. botryoides	29[e]	Red[e]	Large	A	No data found	No data found		Madagascar[b]
F. condensa	20[a]	Green[a]	Large	B		OW bats		Borneo[a]
F. congensis	25[g]	Red[g]	Large	A	No data found	No data found		Africa[a]
F. conocephalifolia	28.5[g]	Red[g]	Large	A		OW bats		New Guinea[b]
F. consociata	12[a]	Red[a]	Large	A	Pisciforms, hornbills, cuculiforms, pigeons, passeriforms	Tree shrews, Asiatic black bear, OW primates, rodents		Peninsular Malaysia[a]
F. copiosa	29[a]	Yellow[a]	Large	B	Cassowary, hornbills, fig parrot	Marsupials, OW bats		New Guinea[b]
F. dammaropsis	60.5[g]	Green[g]	Large	B	Honey eater	Marsupials, OW bats		New Guinea[b]
F. deltoidea	9[a]	Red[a]	Small	A		OW primates		Borneo[a]
F. elastica	≥10[g]	Green[g]	Large	B	Pigeons, passeriforms	OW primates		New Guinea[f]
F. exasperata	9[a]	Red[a]	Small	A	Hornbills, turaco	OW bats, OW primates, artiodactyla		Africa[a]
F. glumosa	9[a]	Red[a]	Small	A	Pisciforms, pigeons, passeriforms	OW primates		Africa[a]
F. grossularoides	10[d]	Red[d]	Small	A	Passerifroms			Borneo[b]
F. hispidioides	35[a]	Green[a]	Large	B		OW bats		New Guinea[b]
F. insipida	40[a]	Green[a]	Large	B		NW primates, NW bats, procyonids, tapirs	Turtle, fish	Venezuela[b]
F. itoana	34[a]	Green[a]	Large	B		OW bats		New Guinea[b]
F. lutea	15[a]	Yellow or red[a]	Large	A or B	Hornbills, pigeons, passeriforms	OW bats, OW primates, elephants		Africa[a]
F. macrophylla	17.5[g]	Purple[g]	Large	A	Cuculiforms, parrots, pigeons, passeriforms	OW bats		Australia[c]
F. maxima	25[a]	Green[a]	Large	B		NW bats, NW primates		Brazil[b]

Table 2 continued

Ficus species	Diameter (mm)	Color	Size code	Fruit type	Avian consumers[h]	Mammalian consumers[h]	Other consumers[h]	Distribution
F. microcarpa	<10[g]	Purple[g]	Small	B	Pisciforms, hornbills, cuculiforms, parrots, pigeons, passeriforms	Marsupials, OW bats, OW primates		New Guinea[b]
F. microdictya	10[a]	Red[a]	Small	A	No data found	No data found		New Guinea[b]
F. mollior	16[a]	Green[a]	Large	B	No data found	No data found		Australia[c]
F. mucuso	40[g]	Orange[g]	Large	B	Pigeons	OW bats, OW primates, elephants		Africa[a]
F. natalensis	13[g]	Red[g]	Large	A	Pisciforms, hornbills, turacos, pigeons, Passeriforms	OW bats, OW primates, artiodactyla, elephants		Africa[a]
F. nodosa	28[a]	Green[a]	Large	B	Hornbills	Marsupials, OW bats		New Guinea[b]
F. nymphaeifolia	22[a]	Green or red[a]	Large	A or B		NW bats		South America[a]
F. parietalis	15[a]	Red[a]	Large	A	Pisciforms, hornbills, passeriforms	OW primates, rodents		Brunei[c]
F. pellucidopunctata	9[a]	Purple[a]	Small	A	Pisciforms, hornbills, trogoniforms, pigeons, passeriforms			Borneo[b]
F. pertusa	14[a]	Yellow or red[a]	Large	A or B	Guans, pisciforms, coraciforms, trogoniforms, parrots, passeriforms	Marsupials, NW bats, NW primates, procyonids		Venezuela[b]
F. phaeosyce	9[g]	Red[g]	Small	A	Cassowary, hornbills, pigeons, passeriforms	Marsupials, OW bats		New Guinea[b]
F. prasinicarpa	7[a]	Red[a]	Small	A	Parrots			Solomon Islands[b]
F. punctata	49[a]	Red[a]	Large	A		OW primates, rodents		Borneo[b]
F. pungens	7[g]	Red[g]	Small	A	Hornbills, parrots, pigeons, passeriforms	Marsupials, OW bats		New Guinea[b]
F. racemosa	25[e]	Green or red[e]	Large	A or B	Galliforms, pisciforms, hornbills, cuculiforms, parrots, pigeons, passeriforms	OW bats, OW primates, bears, civet, artiodactyla, rodents		Australia[b]
F. religiosa	10[g]	Purple[g]	Small	A	Pisciforms, hornbills, cuculiforms, pigeons, passeriforms	OW bats, OW primates, artiodactyla		Lebanon[c]
F. robusta	75[g]	Green[g]	Large	B	No data found	No data found		New Guinea[b]
F. rubiginosa	12.5[g]	Green[g]	Large	B	Cuculiforms, parrots, pigeons, passeriforms	OW bats		Australia[c]

Table 2 continued

Ficus species	Diameter (mm)	Color	Size code	Fruit type	Avian consumers[h]	Mammalian consumers[h]	Other consumers[h]	Distribution
F. ruginerva	37.5[d]	Orange[d]	Large	B	Hornbills	OW primates, rodents		Borneo[b]
F. schumacheri	5[a]	Green[a]	Small	B	No data found	No data found		French Guyana[c]
F. semivestita	33[f]	Green[f]	Large	B		OW bats		New Guinea[b]
F. septica	30[a]	Green[a]	Large	B	Coraciiform, pigeons, passeriform	Marsupials, OW bats, OW primates, rodents		New Guinea[b]
F. subgelderi	12[d]	Orange[d]	Large	A	Pisciforms, hornbills, parrots, pigeons, passeriforms	Tree shrew, OW primates, rodents		Brunei[c]
F. superba	20[g]	Purple[g]	Large	A	Galliforms, pisciforms, hornbills, cuculiforms, parrots, pigeons, passeriforms	OW bats, OW primates, civet, rodents		Borneo[b]
F. sur	19[a]	Red[a]	Small	B	Pisciforms, hornbills, turacos, pigeons, passeriforms	OW bats, OW primates, artiodactyla		Africa[a]
F. sycomorus	26[a]	Yellow[a]	Large	B	Pisciforms, hornbills, turacos, pidgeons, passeriforms	OW bats, OW primates, civet, hyrax		Africa[a]
F. tinctoria	14[a]	Red[a]	Large	A	Hornbills, parrots, pigeons, passeriforms	Marsupials, OW bats, OW primates, rodents		New Guinea[b]
F. trachypison	7[a]	Red[a]	Small	A	Hornbills, parrots, pigeons, passeriforms	Marsupials, OW bats		New Guinea[b]
F. uncinata	22[a]	Red[a]	Large	A		Rodents		Brunei[c]
F. vallis-choudae	33[a]	Yellow or red[a]	Large	B	Pigeons	OW bats, OW primates		Africa[a]
F. variegata	27[a]	Green or red[a]	Large	A or B	Cassowary, hornbill, parrots, passeriforms	Marsupials, OW bast, OW primates, rodents		Australia[b]
F. virens	8[a]	Yellow[a]	Small	A	Cassowary, pisciforms, hornbills, cuculiforms, parrots, pigeons, passeriforms,	OW bats, OW primates, carnivores, rodents		New Guinea[b]
F. virgata	8[a]	Orange[a]	Small	A	Cuculiform, pigeons, passeriforms	OW primates		New Guinea[b]
F. wassa	10[a]	Red[a]	Small	A	Cassowary, hornbill, parrots, pigeons, passeriforms	Marsupials, OW bats		New Guinea[b]
F. xylophylla	25[a]	Orange[a]	Large	A	Pigeons, passeriforms			Brunei[c]
F. xylosycia	13[a]	Red[a]	Large	A	Pigeons, passeriforms			New Guinea[b]

Table 2 continued

Ficus species	Diameter (mm)	Color	Size code	Fruit type	Avian consumers[h]	Mammalian consumers[h]	Other consumers[h]	Distribution
F. yoponensis	18[a]	Green[a]	Large	B	Passeriforms	NW bats, NW primates, procyonids, artiodactyla, rodents		Panama[a]

Superscripts next to the *Ficus* species indicate the source from which data on fruit size and color were obtained. The column "Diameter code" indicates whether the species was classified as small or large based on the 10 mm cutoff point described in the text. The column "Fruit type" indicates whether the species fell in the Type A or B color class based on Janson's (1983) classification, as explained in the text. Data on animal consumers was obtained mainly from Shanahan et al. (2001), and secondarily from fieldwork by S. Lomáscolo. The superscript next to the distribution indicates the source from which this information was obtained. Superscripts denote the source from which the information was obtained: [a]Dominy et al. (2003); [b]Weiblen (2000); [c]Jousselin et al. (2003); [d]Laman and Weiblen (1998); [e]Missouri Botanical Garden website, at http://mobot.mobot.org/W3T/Search/vast.html; [f]G. Weiblen, personal communication; [g]S. Lomáscolo, unpublished data; [h] Shanahan et al. (2001)

OW old world, *NW* new world

included in our study. The technology needed to quantify reflectance in the field has only recently become available (Altshuler 2001, 2003).

Another limitation of this study is the oversimplification of fruit traits and disperser guilds considered to define the syndromes. Fruit size and color were the only fruit traits readily available in the literature and easily comparable among studies, and hence we were unable to include traits such as odor, location on the tree, texture, crop size, among various traits that we acknowledge to be potentially important for frugivores to find fruits (Dominy 2004; Dominy et al. 2001). As we included only color and size, a broad definition of disperser guilds was needed, as it was not feasible to define disperser guilds more narrowly on the basis of their potential selective pressure on just fruit color and size. Despite the differences between mammalian frugivores such as bats, rodents, or primates, we consider it safe to assume that, because most frugivorous mammals can manipulate fruits using their forelimbs and teeth, and because they are on average larger than birds, mammals should generally be able to consume larger fruits than birds can. As for color, despite minor differences in avian color receptors (Bowmaker et al. 1997; Das et al. 1999), birds have a much more complex visual system than mammals do and, in general, a higher variety of cone cells, which are responsible for color vision (Bennett and Thery 2007; Bowmaker et al. 1997; Bowmaker and Hunt 2006). Hence, we consider it a safe assumption that birds can exert a stronger selective pressure on fruit color than mammals can, which are often nocturnal and have fewer color receptors in their retina. Considering only fruit color and size and broadly defined disperser guilds makes this a conservative test of the dispersal syndromes hypothesis, and the patterns of correlated evolution that we found were identified *despite* this generalization and not *because* of it.

Another drawback in our definition of disperser guilds is that we did not incorporate the potential characteristics of the paleofauna that figs have historically interacted with. *Ficus* seems to date back at least 60 million years (Rønsted et al. 2005) and, since then, they have most probably interacted with frugivores that differ from today's frugivores, at least in body size (e.g., extinct New World megafauna, Janzen and Martin 1982). However, we do not have any reason to believe that historic birds and mammals exerted a selective pressure that was significantly different from that of today's birds and mammals, and any discussion on this would be highly speculative. Even with this drawback, we believe that our conclusions remain solid because we predicted how today's birds and mammals should affect the evolution of fruits, and our results in general support our predictions. We think that one important contribution that results from this approach is the generation of readily testable hypotheses about which frugivores are predicted to consume each type of fig. For example, it can be predicted that the figs or *Ficus virgata*, *F. phaeocyse*, and *F. benjamina*, bearing type A colors and classified in the "small" size category, should be dispersed most commonly and efficiently by birds, whereas *F. botryocarpa, F. copiosa*, and *F. damaropsis*, all of which bear Type B colors and are in the "large" size category, should be dispersed most commonly and efficiently by mammals. Qualitative data from a published source (Shanahan et al. 2001) and fieldwork (S. Lomáscolo, unpublished data) on fruit removal by animals for most fig species in our study show that most figs are consumed by many different frugivores, including birds and mammals (Table 2). At first sight, this may seem to contradict the importance of any particular group of frugivore in shaping the evolution of fruit traits. However, the data on Table 2, although highly informative, should be taken with caution because it lacks

indispensable information to assess the potential of a frugivore as a selective force, such as removal rate, gut treatment, deposition of seeds, and overall frugivore effectiveness (sensu Schupp 1993). That said, some general patterns worth investigating further do arise from Table 2. For example, large fruits with contrasting colors, a combination that seemingly falsifies the dispersal syndromes hypothesis, seems to mostly occur in the Old World tropics, where larger birds such as hornbills and cassowaries occur. These may be the exceptions that confirm the rule. However, more data from the New World tropics is needed to confirm this observation. We encourage the generation of quantitative data, such as that mentioned above, to see whether our findings match what is found in nature.

Taken together, our results suggest that differences among taxa of seed dispersers have influenced the evolution of fruit traits. In particular, we conclude that the correlated evolution of fruit size and color support the dispersal syndromes hypothesis.

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