Dispersers shape fruit diversity in *Ficus* (Moraceae)

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Seed dispersal by vertebrates is one of the most common and important plant–animal mutalisms, involving an enormous diversity of fruiting plants and frugivorous animals. Even though plant reproduction depends largely on seed dispersal, evolutionary ecologists have been unable to link co-occurring traits in fruits with differences in behavior, physiology, and morphology of fruit-eating vertebrates. Hence, the origin and maintenance of fruit diversity remains largely unexplained. Using a multivariate phylogenetic comparative test with unbiased estimates of odor and color in figs, we demonstrate that fruit traits evolve in concert and as predicted by differences in the behavior, physiology (perceptive ability) and morphology of their frugivorous seed dispersers. The correlated evolution of traits results in the convergence of general appearance of fruits in species that share disperser types. Observations at fruiting trees independently confirmed that differences in fig traits predict differences in dispersers. Taken together, these results demonstrate that differences among frugivores have shaped the evolution of fruit traits. More broadly, our results underscore the importance of mutalisms in both generating and maintaining biodiversity.

dispersal syndromes | fruit evolution | phylogenetic comparative analysis | plant–animal interactions | seed dispersal

Because most terrestrial plants are rooted in place, seed dispersal is a critical phase of their life cycle (1–3). Plants that fail to disperse are unlikely to reproduce (2–4) and may become endangered (5–9). Thus, seed dispersal is geographically widespread and evolutionarily common (10). For example, up to 90% of woody plants in tropical forests disperse via consumption of fruits and subsequent defecation of their seeds by birds and mammals; these plant species produce an enormous variety of fruits that attract an equally wide variety of seed-dispersing frugivores (11). Given large differences in how frugivorous taxa perceive fruits and disperse seeds and similarly large differences in fruit traits among plant taxa, many studies have hypothesized the existence of "dispersal syndromes"—co-occurring sets of fruit traits matched to the behavior, physiology, and morphology of different types of frugivores (12–15). In fact, these syndromes often are called upon to demonstrate how frugivores have shaped the evolution of fruit traits (12, 16, 17).

Despite the intuitive appeal of dispersal syndromes, their existence has become increasingly controversial because studies that use phylogenetic methods have failed to detect them (18–20), with the possible exceptions of one or two isolated traits (13, 21). This failure raises a paradox: Frugivores strongly affect plant fitness (2, 4) but do not appear to be strong selective agents in driving the evolution of fruit traits (10, 13). Here we test for the existence of bird- and bat-dispersal syndromes in plants of the genus *Ficus* (Moraceae), commonly known as figs.

Figs provide an excellent study system for testing the occurrence of dispersal syndromes because their syconia (hereafter "fruits") are highly variable (Fig. 1) and are consumed by almost all taxa of terrestrial vertebrates (22). Figs depend on frugivores for seed dispersal and, conversely, many frugivores depend on figs as a staple food source (23, 24). Figs are especially diverse in Papua New Guinea (25–27), where they support one of the highest diversities of frugivores and where we conducted our study.

Because birds have a relatively narrow gape, lack teeth, have tetrachromatic vision (28, 29) and a poor sense of smell, and usually take fruits while perched, we predict that bird-syndrome fruits will be small, soft, visually conspicuous (red, purple or black, and contrasting against background), nonodorous, and presented amid foliage along branches (12, 14, 15). On the other hand, because bats have teeth and can eat fruits piecemeal, are mostly nocturnal (limiting their perception of color), have a keen sense of smell (30–32), and usually take fruits in flight or after crawling to reach them (16, 33), we predict that bat-syndrome fruits will be relatively large, hard, visually inconspicuous (green, yellow, pale orange, or brown, and noncontrasting against background), odorous, and presented along the trunk (cauliflorous) or away from foliage (16, 17). Hence, if birds and bats are important in shaping the evolution of fruits, these fruit characteristics should evolve nonrandomly, converging differently in bird-dispersed and bat-dispersed species. Unlike previous studies that tested for correlated evolution between individual fruit traits and type of disperser (13), we tested for correlated evolution among several fruit traits known to be important for birds and bats to find and consume fruits. Furthermore, we verify the dispersal syndromes in the field by linking them to observed consumption of fruits by birds and bats.

Results

By quantifying fruit characteristics that are important for frugivores in finding and consuming fruits, documenting fig consumers through nondisruptive video-recording at fruiting figs in a natural setting, and analyzing all fruit traits simultaneously while controlling for phylogenetic effects, we found that fruit characteristics indeed evolve nonrandomly and as predicted by selection by frugivores. Evolutionary principal components analysis (EPCA), a multivariate analog of the well-established technique of phylogenetically independent contrasts (34), revealed that small fruits are soft, reflect more in the red and UV/blue part of the spectrum than in the green part of the spectrum, are darker, and are highly contrasting against the background (Fig. 2). Also, they produce fewer volatile compounds and grow among the foliage, on the branches. Large fruits, on the other hand, tend to be green or yellow, light in color, and less contrasting against the background. They emit more volatile compounds and are cauliflorous and exposed away from the foliage (Fig. 2). These combinations of characteristics, which cluster independently of phylogeny, correspond to the predicted bird and bat syndromes, respectively. They explain 42% of the overall variation in fruit traits, as summarized in the first two axes of the EPCA (Fig. 2 and Tables S1 and S2).

Data on frugivore visits to 35 fig species show that figs with traits predicted by the bird-dispersal syndrome are indeed dispersed by
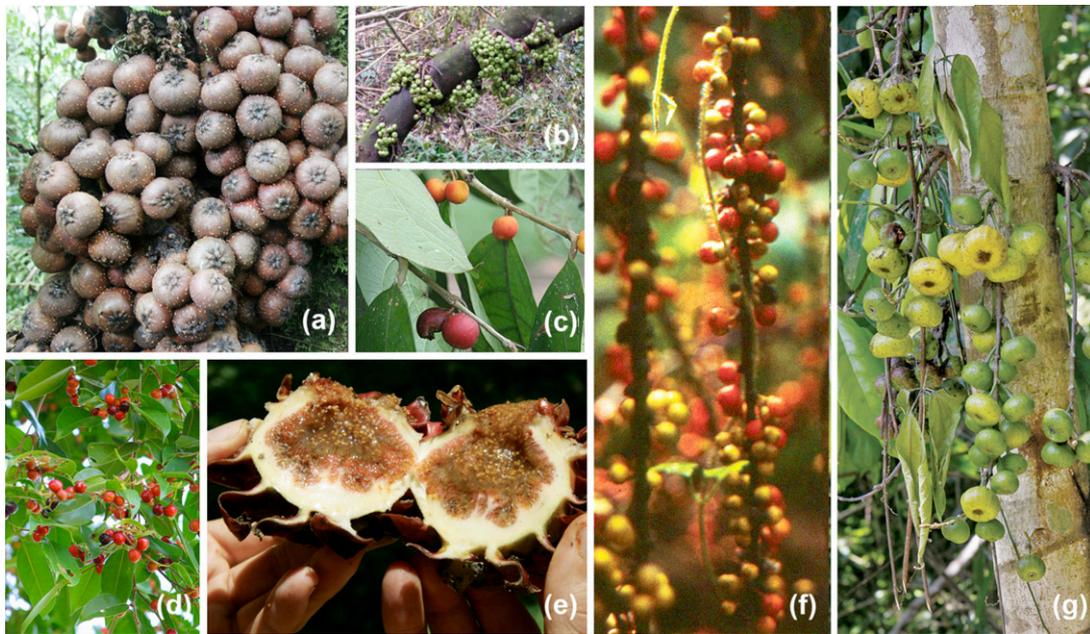
Fig. 1. Diversity of fruit characteristics in some figs from Papua New Guinea. (*A*) *Ficus pachyrrachys,* mean diameter (x̄) = 59.4 mm. (*B*) *F. hispidoides*, x̄ = 52.1 mm. (*C*) *F. subulata*, x̄ = 11.3 mm. (*D*) *F. benjamina*, x̄ = 9.8 mm (*E*) *F. dammaropsis*, x̄ = 69.5 mm. This is a fig cut in half to show the numerous tiny seeds. (*F*) *F. pungens,* x̄ = 9.0 mm. (*G*) *F. congesta,* x̄ = 40.8 mm. *A, B,* and *G* show the typical bat-syndrome traits. *C, D,* and *F* show the typical bird-syndrome traits.

birds, figs with traits predicted by the bat syndrome are dispersed by bats, and figs with intermediate phenotypes are dispersed by both birds and bats. These data come from video recording at fruiting trees (1,019 h, including night observations with infrared illumination) and from the literature (22). Overall, nine fig species were dispersed exclusively by passerine birds, 14 by bats (flying foxes), and 12 by both birds and bats ("mixed" dispersers) (Fig. S1 and Table S1). Importantly, the distribution of fig species observed to be dispersed by birds, bats, and mixed assemblages on the EPCA axes is not random and matches the distribution of fruit types (syndromes): EPC 1: bird < mixed < bat, Jonckheere-Terpstra statistic = 131, $P = 0.017$; EPC 2: bird > mixed > bat, Jonckheere-Terpstra statistic = 269, $P = 0.020$.

Discussion

Our results show that at least six fruit traits important in attracting frugivores (16, 17, 35, 36) evolved in concert (i.e., into syndromes) across different clades of figs. These sets of fruit traits match those predicted by morphological, physiological (sensory), and behavioral differences in the figs' dispersers, supporting the hypothesis that natural selection by frugivores is an important driver of variation in fruit traits. This result is fundamentally different from those of other studies, in which authors addressed the evolution of individual fruit traits associated with different types of frugivores (13). Although other hypotheses also can explain the correlated evolution of a couple of fruit traits (18, 37–39), only the dispersal-syndrome hypothesis can account parsimoniously for the correlated evolution of so many traits.

The dispersal-syndrome hypothesis is supported further by our field observations of dispersers in fruiting trees: Fig species with bird-syndrome traits were dispersed primarily by birds, whereas fig species with bat-syndrome traits were dispersed primarily by bats. Taken together, our results demonstrate that frugivores are indeed important in determining the evolution of fruit traits in the genus *Ficus* and suggest that they are at least partially responsible for the immense diversity of fleshy fruits produced by figs and other animal-dispersed plants, worldwide. However, our EPCA explained only about half of the variation in fig traits, suggesting that other factors also drive the evolution of fruit traits. Studies on

other taxa with highly variable fruit types (e.g., *Solanum*, *Eugenia*, palm trees) may be necessary to reveal these additional agents of selection on fruiting plants.

To what extent can our results be generalized to the entire genus of *Ficus*? With one exception (21), no other phylogenetic comparative analyses exist for this genus. In that study, Lomáscolo et al. (21) found that at least two characteristics we have shown to be important in frugivore attraction (fruit color and size) also evolve in correlation, as expected by natural selection from avian and mammalian frugivores. Unlike this study, which focused on an unusually high diversity of figs in a single area, the previous study incorporated fig species from around the world (Africa, Australia, Borneo, Brazil, China, Madagascar, Malaysia, New Guinea, and Venezuela). Although not in a phylogenetic context, Lomáscolo et al. (21) found that the same correlation we found between fruit color and size also was apparent for an even larger and geographically diverse set of fig species. Furthermore, we emphasize that the species included in the present study do not fall within a single clade of *Ficus* but instead are widespread in larger phylogenies of 146 (40) and 100 (41) species. This variety of species suggests the pattern we observed likely occurs across a larger portion of the genus than we observed in Papua New Guinea.

Methodological limitations may have prevented previous studies from finding strong support for the dispersal-syndrome hypothesis. In contrast to previous studies, we controlled for nonindependence of species by using a molecular phylogeny at the species level; eliminated human biases in quantifying color and odor; used internally consistent frugivore taxa, i.e., taxa whose members are similar in the way they sense the world and process fruits and, hence, exert similar selective pressure; and analyzed all traits simultaneously, an approach that likely approximates the holistic manner in which frugivores find and discriminate different types of fruits. Finally, we focused on a tropical plant genus that, with immense variation in fruit traits and a pan-tropical distribution, is exposed to an unusually wide variety of dispersers.

Our results are relevant to conservation biology because species of frugivores and fruiting plants are being lost at an alarming rate (42, 43), each loss representing one less mutualistic partner. We have shown that these mutualisms play a key role in generating

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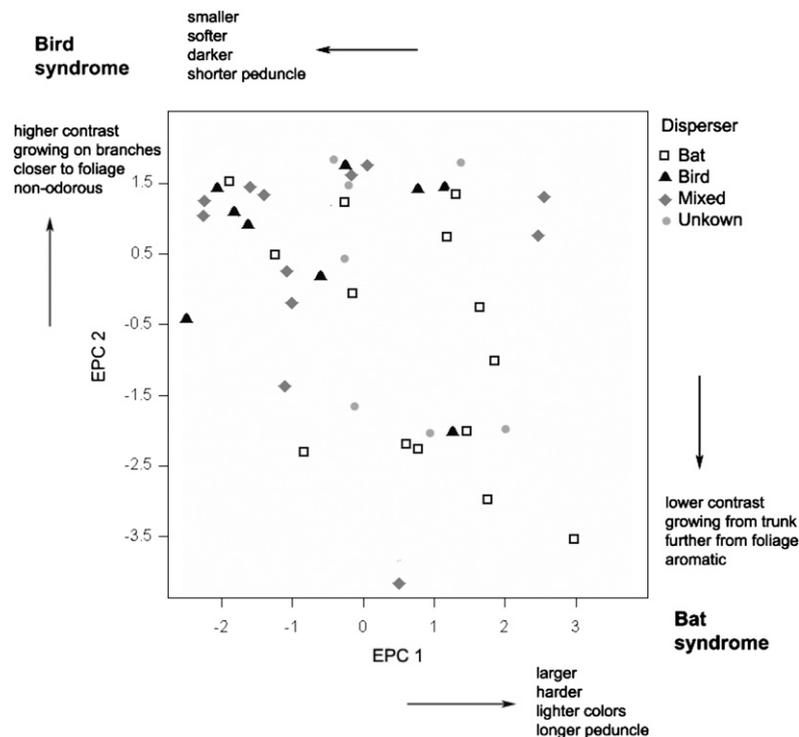
Fig. 2. Ordination of 42 co-occurring fig species with EPCA. According to the loadings of all variables on EPC 1 and 2 (Table S2), fig species plotted in the upper left corner bear bird-syndrome trait combinations, and species in the lower right corner have bat-syndrome combinations. Hollow black squares: bat-dispersed figs; solid black triangles: bird-dispersed figs; solid gray diamonds: figs dispersed by birds and bats; solid gray circles: species with unknown dispersers. Species identification is given in Table S1.

biodiversity, not simply in maintaining it. This distinction between ecological importance (i.e., the role of seed dispersers in plant reproduction) and evolutionary importance (i.e., the role of seed dispersers in diversification of fruit types) is often overlooked in conservation biology (44–46). Ultimately, the loss of mutualists has a double impact—first, an immediate reduction in biodiversity, and second, the loss of an evolutionary process that produced the mutualism and initially created the unique combinations of traits that we see today.

Methods

Study Site. Fruit trait data were collected in the field in a lowland forest near the village of Ohu and on the coast and nearby islands of Madang city (145° E, 5° S) in Madang Province, Papua New Guinea. Approximately 151 species of figs exist in the country (27), and 54 species exist in our study site (26).

Fruit Trait Data. Fruits were collected from two to eight individuals per species (average 3.1 individuals/species), with the exception of seven species for which only one individual was found (Table S3). Within-species variation in fruit traits was negligible compared with between-species variation, so we decided to include the species with data for only one individual. Within 3 h of collection, color was quantified using a spectrometer, and volatile compounds were extracted using a vacuum pump. Color variables were brightness, reflectance in the green, red, and UV/blue part of the spectrum (*SI Text*, Fig. S2), and chromatic contrast against the background (Fig. S3). See *SI Text* for analysis using alternative color variables (brightness, hue, and chroma) (Figs. S4 and S5, Table S2). We also measured fruit diameter at the widest point (Fig. S3), softness using a four-point ordinal scale, distance from nearest leaves, and peduncle length. At collection, we noted whether the fruit was cauliflorous or grew on branches amid foliage.

We quantified fruit color using a USB2000 portable spectrometer and a PX-2 pulsed xenon light source (Ocean Optics), which took accurate readings between 300 and 740 nm, a spectrum including the UV range. We scanned fruits using a sensor with six optical fibers illuminating the target fruit and a seventh fiber that returned the reflected light to the spectrometer. The scanning angle was fixed at 45° by using a black metal stand with a hole positioned at that

angle. The metal stand also blocked external light. To keep the distance between the end of the sensor and the fruit constant, we fastened a non–UV-filtering microscope slide to the opening of the hole where the sensor was introduced. We scanned three different spots on each fruit. We calculated reflectance as the proportion of a Spectralon white reflectance standard (Labsphere); therefore reflectance measures are unitless. The three spectra for each species were averaged every 5 nm. Color contrasts were calculated as the Euclidean distance between the color of a fruit and the color of the structure (leaves or bark, depending on the species) against which it was exposed to the frugivore. Spectra were normalized to the same brightness by dividing the reflectance at each wavelength by the total reflectance for each species. This computation generates contrasts caused by the color of the fruits, not to their brightness. These contrasts are called "chromatic" contrasts (35) and are calculated as

$$D = \sqrt{\sum [Qf(\lambda) - Qb(\lambda)]^2}$$

where Qf is the color spectrum of the fruit, and Qb is the color spectrum of the background structure; λ is the wavelength in nm; and the sum corresponds to the complete spectrum between 300–740 nm.

To capture volatile compounds, we collected fruits in the field, brought them to the laboratory, and placed them inside bags made of inert material (Reynolds oven baking bags). A vacuum pump (Welch model #2522B-01) sucked air at about 1 L/min through a carbon filter into the bag and through a Super Q-filled filter (Alltech) at the opposite end, which adsorbed volatiles emitted by the fruits. For a given collection of fruits in a bag, volatiles were collected continuously for 4 h, beginning no more than 3 h after the fruits had been collected from the tree. We extracted the trapped volatiles with methylene chloride and separated and quantified them using gas chromatography with flame ionization detection (Agilent 5890). We also identified some of the main compounds that seemed to be important in discriminating bat-dispersed versus bird-dispersed fruits (*SI Text*). Because there were too many compounds (n ~119) to identify or analyze individually, and because we were most interested in determining whether bird-dispersed and bat-dispersed fruits differ in overall production of volatile compounds, we calculated the total amount of volatile compounds by summing the areas under each compound's peak for each species. We standardized for number

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and size of fruits in a bag by dividing the amount of each volatile contained in the sample by the total surface area of the fruits in the bag.

Seed-Disperser Data. All data on fig consumption come from ripe figs that contained viable seeds, commonly called "seed figs" (47). We recorded consumption of figs using video cameras (Sony DCR-HC40), positioned 3–5 m away from fruiting *Ficus* trees, pointed at the tree crown, and visited only to change tapes or batteries. To record both diurnal and nocturnal frugivores, we started videotaping at 0600 hours and ended at 1030 hours then continued videotaping between 1830 hours and 2300 hours. We recorded nocturnal visits using an infrared light (Sony HVL-IRH2). We recorded between one and four individuals of 29 species of figs for a total of 1,019 h. Unless a frugivore remained in view and did not consume fruit while in a fruiting tree, we considered it a seed disperser of that fig species. We observed frugivores at 16 species of figs. We were able to include an additional 19 species in our analysis by extracting data on frugivore visitation to figs provided in a previous study (22). For eight of these fig species we found conflicting information between the dispersers that we observed and those reported by the previous study. We ran duplicate analyses, first using disperser data for these eight species from our study and then using the data from the previous study. Because the results were qualitatively similar, we report here only the results based on our own observations (results with alternative classification are given in *SI Text*).

Phylogeny Reconstruction. We reconstructed the phylogeny for the 42 *Ficus* species included in this study using DNA sequences from GenBank. We used a maximum likelihood criterion, which searches for the phylogenetic tree that best fits the data, given a particular model of evolution. We decided upon the model of evolution that best described our data using the Akaike Information Criterion (AIC) from the model set examined using MODELTEST 3.6 (48). We conducted a heuristic search in PAUP* with 10 random sequence additions using the appropriate model (GTR+G) and parameters recommended by MODELTEST 3.6 (48). We assessed support on the phylogeny with 500 maximum likelihood bootstrap replicates using GARLI 0.951 (49). Unresolved nodes were resolved for further comparative analyses based on published phylogenies (40, 41, 50, 51). We resolved randomly the few cases in which species were not included in any published phylogeny (*F. adelpha, F. ampelas,*

F. macrorhyncha, F. morobensis, F. pacchyrachis, F. ternatana, and *F. trachypison*). Several random combinations were tested, and all yielded similar results, so all results reported are based on one resolution (Fig. S4).

Statistical Analyses. The phylogenetic comparative analysis was done using EPCA, which is similar to Principal Components Analysis (PCA) in that it finds orthogonal axes that explain the greatest variation in the data. Unlike PCA, however, EPCA uses a matrix of evolutionary transitions as variables. Evolutionary transitions of each variable are calculated as the change in trait value from the time each species split from its nearest ancestor node (Fig. S5) (52). The patterns of association revealed by EPCA can be attributed to the action of natural selection rather than solely to evolutionary history (52–54). EPCA scores of bird-, mixed-, and bat-dispersed species were compared using a Jonckheere-Tepstra test of ordered hypotheses (55, 56).

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