

THE ROLE OF FRUGIVORES IN THE EVOLUTION OF FRUIT IN *FICUS* (MORACEAE)

By

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To my husband and son, who are the compass that keeps me focused on the true North of my life.

To my parents, for just giving, from the very beginning.

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We attempt to resolve a central controversy of plant-animal interactions: whether seed dispersers are an important factor in shaping the evolution of fruit traits. By focusing on a genus (*Ficus*) with extremely variable fruit traits, we could study fruit variation using a phylogeny to control for the evolutionary non-independence of fig species. We studied six characters that were hypothesized to most likely influence a frugivore's ability to find and access fruits: color, odor, size, softness, exposure away from foliage, and location on the tree. This is the first study on the role of seed dispersers in fruit evolution to combine quantitative measures of color and odor in a detailed phylogenetic comparative analysis. We took a closer look at fruit color because it is probably the first trait mediating fruit-frugivore interactions and because it has historically been mismeasured or ignored in studies of fruit trait evolution. We asked: (1) Can frugivores discriminate between different colors? (2) Have fruit colors of plants that share dispersers converged to a common signal? (3) Do fruit characters co-occur in non-random patterns among species of *Ficus*? (4) If they do, are the patterns most likely the result of phylogeny or selective pressure by different types of frugivores?

Results show that color can be used as a signal by frugivores because it can be discriminated by birds and primates, two of the most common frugivores worldwide. Fruit color

has converged in distantly related species, which can be predicted under selective pressure by frugivores. However, we cannot discard the role of microbes as a selective force that determines the amount of the pigment anthocyanin, which has antifungal properties. Color, especially brightness and contrast against the background, have evolved in correlation with fruit odor, size, softness, and exposure away from leaves. Results show that the correlation among these traits cannot be explained solely by phylogeny: a large portion of the variation in fruit traits is adaptive. The most likely explanation for the correlated evolution of six of the traits studied here is selection by frugivores. This pattern holds for figs in one geographic region and worldwide, and that it holds both within and between fig species. Taken together, this evidence argues for the prevalence of seed dispersers over phylogeny as the main driver of the evolution of fruit traits in figs.

CHAPTER 1 GENERAL INTRODUCTION

“It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us.”

Darwin, 1859, On The Origin of Species

Species interactions are ubiquitous and important in shaping many ecological and evolutionary processes, as depicted by the paragraph by Darwin cited above (1859). All biologists will probably agree on the fact that every system we study is affected in one way or another by the interaction among and within species. What we might not all agree on, however, is what types of species interactions are most important (Bertness et al. 1999; Bruno et al. 2003). Negative interactions have received the most attention by ecologists, which is illustrated by two facts. First, more studies have been published on negative interactions, including competition, predation and herbivory, than on positive interactions, such as pollination, seed dispersal and facilitation (~146,000 and ~27,000, respectively, as resulting from a Web of Science search using the name of the interactions mentioned above together with “ecology” as keywords). And second, research on negative interactions have had stronger incidence in ecological theory, as illustrated by some of the most used models in ecology, namely, Lotka and Volterra’s interspecific competition model, Gause’s competitive exclusion principle, and Hutchinson’s and MacArthur’s work on the niche and species packing (Bruno et al. 2003). Positive interactions have been largely overlooked, although they have an important role in several ecological

processes, such as facilitation through habitat modification (Bertness and Leonard 1997; Halpern et al. 2007), and plant reproduction through pollination and seed dispersal. This leads to the focus of this study, which is the positive interaction between fleshy-fruited plants and the animals that eat the fruits and disperse the seed, potentially aiding in plant reproduction.

The field of seed dispersal is wrestling with a major contradiction: although ecological evidence demonstrates that frugivores are important for the reproduction of fruiting plants, any evolutionary role of seed dispersers in shaping fruit characteristics is difficult to detect. Those who believe in the evolutionary importance of seed dispersers in shaping fruit evolution point to the existence of apparently non-random combinations of fruit traits that match the sensory abilities of the frugivores that feed on them (Janson 1983; Kalko et al. 1996; Korine et al. 2000; Lord et al. 2002). These studies argue that frugivores must have selected for these suites of traits, which are referred to as dispersal syndromes. On the opposite side of the issue are those who argue that other factors, such as phylogeny or physiological constraints, play a more prevalent role than seed dispersers in shaping fruit traits (Fischer and Chapman 1993; Herrera 1985; 1987; 1992). They explain the match between fruit traits and frugivores' sensory abilities as preexisting preferences of frugivores for certain types of fruits: the frugivores will feed on the fruits that are most easy to find and accessible to them. This process is called "ecological fitting" (Janzen 1980; 1985).

We suggest that much of this controversy stems from the disparate approaches of different studies, the qualitative measurements of fruit traits (especially color and odor), and the lack of consistency among studies in the fruit traits that are argued to be important for frugivores to find and access fruit. Studies that have claimed an association between fruit traits and seed dispersers have generally not controlled for phylogenetic effects and hence attribute any similarity in fruit

traits among species dispersed by similar frugivores to common selective forces by those frugivores; they tend to disregard the possibility that a group of species might bear similar fruits simply because they have shared a common ancestor (Felsenstein 1985; Harvey and Pagel 1991). Studies that have claimed a lack of association between fruit traits and seed dispersers have either focused at high taxonomic levels and consequently have ignored great variation at the species level (Fischer and Chapman 1993); have taken place in temperate regions, and therefore not incorporated the diversity of dispersers and fruiting plants in tropical regions (Herrera 1987; 1992); or have neglected traits that may be very important in mediating plant-animal interactions, such as color and odor (Jordano 1995).

We test the Dispersal Syndrome hypothesis, which states that frugivores have selected for the non-random combination of fruit traits found in nature. We study fruit characteristics at the species level in the genus *Ficus*, based on a molecular phylogeny to control for the evolutionary non-independence of species. We focus on morphological characters of fruit that most likely affect frugivore fruit choice, and trace their evolution in the genus *Ficus* (Moraceae) to infer the evolutionary trajectories and underlying causes of the appearance of modern-day figs. We show that fruit color and size seem to have evolved in correlation, as predicted by the Dispersal Syndrome hypothesis (Chapter 2). We then test an important assumption of the Dispersal Syndrome hypothesis, namely that the fruit traits used to define dispersal syndromes can actually be perceived and differentiated by the frugivores that the traits are signaling (Chapter 3). We focus on color, which has been argued to be the most important trait determining fruit choice by frugivores (Voigt et al. 2004) but has been largely mismeasured or ignored in studies of fruit evolution, probably due to the difficulties of quantifying it in an objective way. We specifically test the Signal Convergence hypothesis, which states that if color is a reliable signal to

dispersers, then colors should converge among species dispersed by similar frugivores. We show that frugivores can distinguish the color of fruits they disperse from the color of fruits dispersed by other frugivores. We conclude that color can be used as a signal to dispersers. We also show that colors that attract different frugivores have evolved independently in different genera and families of fruiting plants.

The most important finding of our work is that when seven of the traits hypothesized to be most important for frugivores to find and access available fruits are analyzed together, they suggest that dispersers are an important selective force in determining the diversity of fruiting plants, even when phylogenetic effects are accounted for (Chapter 4). Lastly, a within-species study of dioecious figs comparing individuals with viable seeds and often consumed by frugivores (female plants, called seed figs), with individuals without viable seeds and not consumed by frugivores (male plants, called gall figs), shows the same pattern just described for interspecific comparisons (Chapter 5).

Overall, the results of my study show that seed dispersers have had an important role in shaping the evolution of fruit traits. Other alternative hypotheses that were used to explain the diversity of specific traits such as color, or the correlated evolution of pairs of traits, such as color and size, were not tested, but are discussed. Although some alternative hypotheses cannot be discarded to explain the diversity of specific traits, such as the antimicrobial role of pigments in ripe fruits, only the Dispersal Syndrome hypothesis can account for the correlated evolution of the six fruit traits that we examined.

CHAPTER 2 CORRELATED EVOLUTION OF FIG SIZE AND COLOR SUPPORTS THE DISPERSAL SYNDROME HYPOTHESIS

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, Schaeffer et al. 2006). For example, fruit-eating birds are diurnal, have excellent color vision, a poor sense of smell, and are limited by gape width in the size of fruit they can swallow (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. 2004b). Fruit-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 2002a; McKey 1975; van der Pijl 1969). At least superficially, fruit traits such as size and color seem to be associated non-randomly, 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 co-occurrence of fruit size and color. One we termed the Phylogenetic Inertia hypothesis, which states 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. 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; 1985; Strauss et al. 2006) that does not drive the evolution of fruit traits. Alternatively, the Dispersal Syndrome hypothesis states that 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.

Tests of these hypotheses with phylogenetic comparative methods have commonly rejected the Dispersal Syndrome 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 Syndrome 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 those of 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 Syndrome 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, purple, or black 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 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 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 beak. Through this conservative test of the Dispersal Syndrome 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 Syndrome 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 4mm in diameter to as big as 80mm (Dominy et al. 2003; Laman and Weiblen 1998, S. Lomáscolo pers. obs.). *Ficus* has 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 1274 bird and mammal species in 523 genera, in addition to reptiles and fishes (Shanahan et al. 2001b). 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 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 Syndrome 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 non-phylogenetic comparative analyses). Non-phylogenetic 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 intraspecific 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 interspecific variation for mammal-colored and bird-colored fruits, and compared them using an F-test. This approach tests the results of selective pressure on fruit size by birds and mammals 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, pers. comm.) 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 (10mm) of species included in our study: "small" (≤ 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 pers. comm.; Laman and Weiblen 1998), with the mean fruit diameter and bird gape width reported by Wheelright (1985), and with the largest diameter of fruits typically consumed by some of the most common fruit-eating passerine birds in Costa Rica (Levey 1987).

Occasionally, different sources provided contradictory information about fruit color of a given species. For example, *Ficus asperifolia* is reported to produce yellow or red fruits (Dominy et al. 2003), which means it could be classified either as 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 Syndrome 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 non-conservative reconstruction where, for each ambiguous classification, we chose the color that matched the hypothesis of correlated evolution between the two traits. By comparing results of these two approaches, we are able to assess the sensitivity of studies like ours to sometimes arbitrary

character state reconstruction – a commonly encountered 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. mucoso*, *F. natalensis*, *F. asperifolia*2, and *F. vallis-choudae*), we sequenced the ITS region ourselves. 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 μ L reactions containing 0.4 units of NEB Taq polymerase (New England Biolabs, Beverly, MA) 1.5mM MgCl₂, 0.4 μ M of each primer and 0.1mM 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 12min. PCR products were cleaned with Wizard® SV Gel and PCR Clean-up System (Promega, Maddison, WI) and diluted to approximately 1ng/ μ L for sequencing.

We sequenced all regions in both directions on a CEQ 8000 capillary sequencer (Beckman-Coulter, Fullerton, CA) using $\frac{1}{4}$ reaction volumes with the addition of 80mM Tris and 2 mM MgCl₂ (pH 9) to complete the volume of a full reaction. To edit the sequences manually we used Sequencher TM (V4.1.4, Genecodes, AnnArbor, MI).

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 base pairs 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 base pairs deletions and multiple base pairs deletions that could not be unambiguously aligned were treated as missing data. A low complexity region ranging from 20 to 40 base pairs 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), Jousellin 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 Weiblen's (2000), Jousselin et al.'s (2003), and Rønsted et al.'s (2005) analyses, which were 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 5000 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 1000 bootstrap replicates with 100 SAR within each replicate using TBR.

The maximum likelihood 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 region 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 10 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 1000 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 maximum likelihood 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 non-random 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 the AIC (Akaike's Information Criterion) (Burnham and Anderson 2003). Since results of this test were not conclusive, and since 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 our majority rule tree, Weiblen's (2000), Jousellin et al's (2003), or Rønsted et al's (2005) reconstructions.

Sensitivity analysis: The Concentrated Changes test requires specification of several different algorithms, although defining the algorithms 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 if 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 the conservative and non-conservative trait reconstruction, using color and size as the independent variables.

Non-phylogenetic comparative analysis: To test if type A fruits had smaller diameter than type B fruits (prediction #1), we used a Kolmogorov-Smirnov non-parametric 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 maximum likelihood, 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. 2-1). With taxa shared among our phylogenies and published phylogenies, all the major clades with high bootstrap values were essentially identical. Conflicts occurred only in poorly supported groups and those conflicts were minor. Slight differences in topology between the ML and parsimony tree did not affect the results of our phylogenetic comparative analyses (see below).

Non-Phylogenetic 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 larger than 10mm, 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 $Z = 1.818$; $p = 0.003$). For the entire set of species, the difference was in the same direction but not as large ($\bar{x}_A = 15.46$ mm, $n_A = 56$; $\bar{x}_B = 18.13$ mm, $n_B = 72$; Kolmogorov-Smirnov $Z = 1.359$; $p = 0.050$). The variance in 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 ($\sigma^2_A = 79.63$; $\sigma^2_B = 281.69$; $F_{35,26} = 3.54$; $p < 0.001$). However, this difference in variance was not as large when all species were included in the analysis ($\sigma^2_A = 106.68$; $\sigma^2_B = 116.36$; $F_{71,55} = 1.09$; One tailed test $p > 0.25$) (Fig. 2-2).

Phylogenetic Comparative Analysis

For two reasons, we emphasize the analyses performed on the ML tree (Fig. 2-3). First, Pagel's Likelihood Ratio test (1994) requires branch lengths, which were only available on the ML trees. Second, the parsimony searches came up with over 5000 most parsimonious trees and performing the comparative analyses in all 5000 of them was unwieldy. A preliminary comparative analysis performed on a randomly chosen parsimony tree showed no difference with the comparative analyses performed on the ML tree.

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, which prediction #1, regardless of the coding of ambiguous fruit colors (Conservative reconstruction: likelihood ratio = 4.889; $p = 0.027$; 1000 simulations. Non-conservative reconstruction: likelihood ratio = 4.990; $p = 0.027$; 1026 simulations).

To determine the independent variable for Maddison's Concentrated Changes test (Maddison 1990), assuming color precedes size is more likely (ln likelihood = -80.31) than assuming size precedes 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. 2-3). When color is considered as 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 2-1). When size is considered the independent variable, however, results vary greatly according to the algorithm combination and character state reconstruction used and support for prediction #1 is mixed ($0.04 < p < 0.54$; Table 2-1).

Discussion

Overall, our results support the Dispersal Syndrome hypothesis. Mean diameter is smaller for Type A fruits than for Type B fruits, supporting prediction #1 of the Dispersal Syndrome hypothesis. This pattern holds when using raw data for the subset of species included in our phylogeny and when using raw data for all the species of figs found in the literature. It also holds when examined with phylogenetic comparative methods: fruit size 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 Syndrome 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 Syndrome 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 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.

Returning to prediction #1, an alternative hypothesis for the observed non-random 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 extra photosynthetic tissue of large fruits would be selectively advantageous, 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 different color and similar size.

The sensitivity analysis for Maddison's Concentrated Changes test (Maddison 1990) helped us identify which source of variation has the stronger impact on our results, given uncertainty in both the phylogenetic reconstruction and the mode of fruit trait evolution. The largest impact in 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 strength of the correlation varied widely, especially between the conservative and

non-conservative 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 more confidence that color can be considered the independent variable.

An important caveat of our methodology is that our categorization of colors was based on the perception of colors by humans. Birds, however, see the world differently than humans; they 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 entire 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 included in our study. The technology necessary to quantify reflectance in the field has only recently become available (Altshuler 2001; Altshuler 2003).

Another limitation of this study is the oversimplification of fruit traits and disperser guilds used 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 other traits that may be potentially important for frugivores to find fruits (Dominy 2004; Dominy et al. 2001). Because 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 potentially important differences among different taxa of 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 generally larger than birds, mammals should be able to consume larger fruits than can birds. 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 and, in general, a higher variety of cone cells, which are responsible for color vision (Bennett and They 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 less color receptors in their retina. Considering only fruit color and size and broadly defined disperser guilds makes our analyses a conservative test of the Dispersal Syndrome hypothesis. In short, the patterns of correlated evolution that we found were identified despite this generalization, 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 interacted with historically. Modern day figs appear to date back at least 60 million years (Rønsted et al. 2005) and they have almost certainly 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 selective pressures that were significantly different from those of current birds and mammals, and any discussion on this would be highly speculative. Even with this limitation, we believe our conclusions remain solid because we predicted how current birds and mammals should affect the evolution of fruits, and our results generally supported our predictions.

Our results can be used to generate readily testable hypotheses about which frugivores consume a given type of fig. For example, it can be predicted that the figs of *Ficus phaeocyse* and *F. benjamina*, bearing type A colors and classified in the “small” size category, are dispersed most commonly and efficiently by birds, whereas *F. botryocarpa*, *F. copiosa*, and *F. dammaropsis*, all of which bear type B colors and are in the “large” size category, are dispersed most commonly and efficiently by mammals. Qualitative data from a published source (Shanahan et al. 2001b) and from fieldwork (S. Lomáscolo, unpublished data) on fruit removal by animals for most fig species in our study show that most figs are consumed by a wide diversity of frugivores, including birds and mammals (Table 2-2). At first glance, this may seem to contradict the importance of any particular group of frugivore in shaping the evolution of fruit traits. However, the data in Table 2, although highly informative, should be taken with caution because they do not provide information that would allow one to assess the potential of a given frugivore as a selective force. Such information would include fruit removal rate, gut treatment, deposition of seeds, and overall frugivore effectiveness (sensu Schupp 1993). That said, some general patterns arise from Table 2-2. For example, large fruits often have contrasting colors, a combination that seemingly falsifies the Dispersal Syndrome hypothesis. These species tend to occur in the Old World tropics, where larger birds such as hornbills and cassowaries occur. These may be “exceptions that prove the rule.” We predict that contrasting colored fruits should

be smaller in places where such large frugivorous birds are less common, i.e., in the New World. However, more data from the New World tropics is needed to test this idea. We encourage the collection and application of quantitative data that address disperser effectiveness to determine 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 Syndrome hypothesis.

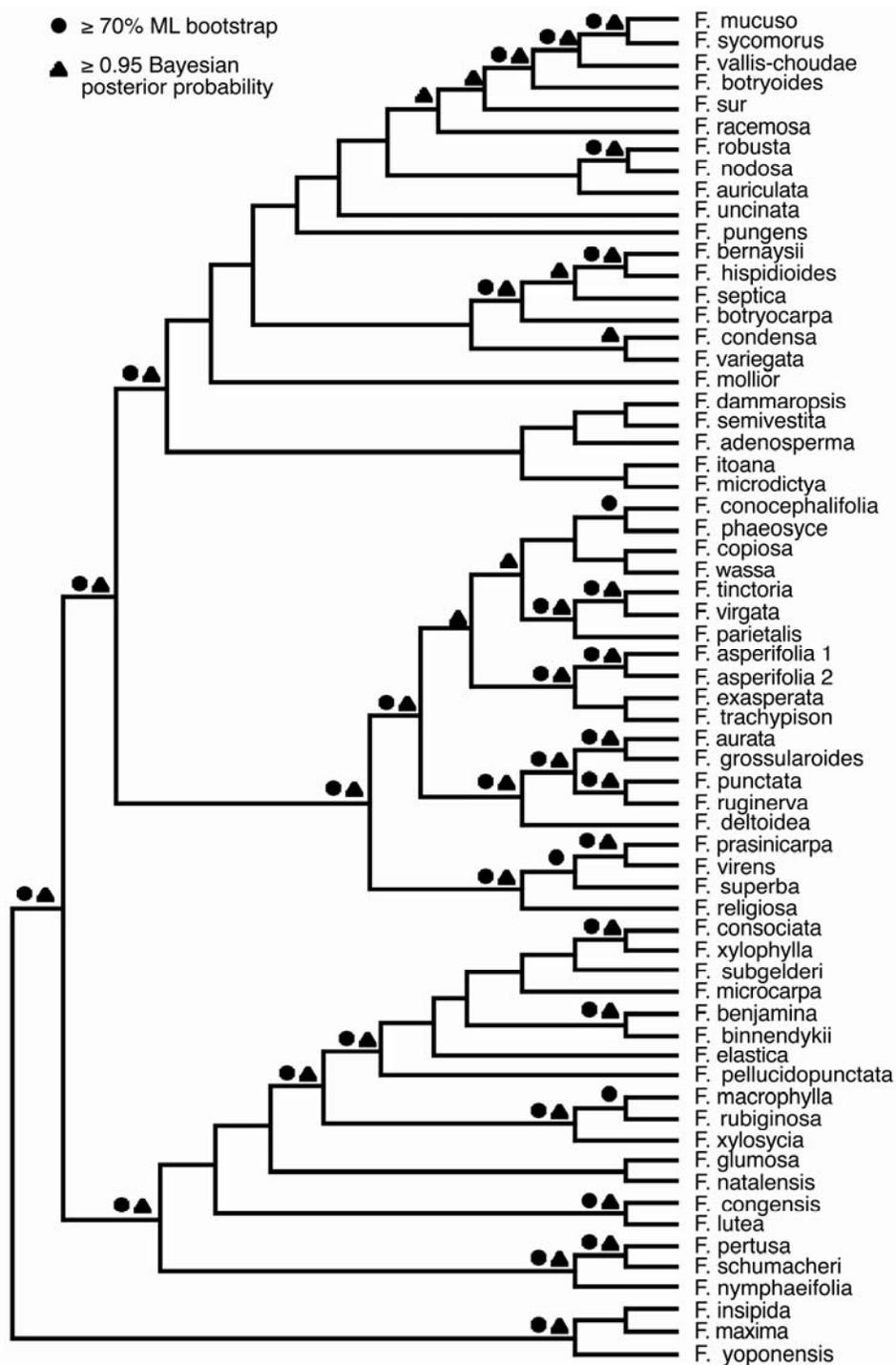


Figure 2-1. Bootstrap support and Bayesian posterior probabilities on the maximum likelihood phylogeny of 64 *Ficus* species used for analyses. Nodes with strong bootstrap support in maximum likelihood analyses (greater than or equal to 70%) are indicated with a circle; nodes with strong support using Bayesian posterior probabilities (greater than or equal to 0.95) are indicated with a triangle

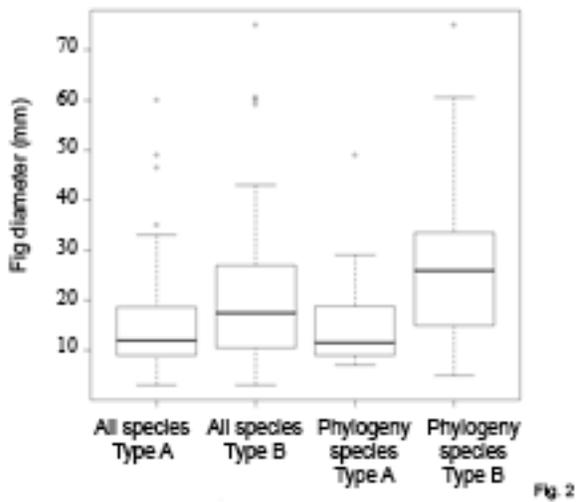


Figure 2-2. Box and whiskers plot comparing the mean diameter of Type A (red, purple) and Type B (green, yellow, brown, and orange) fruits (Janson 1983) for the set of *Ficus* species in our phylogeny ($n_A = 36$, $n_B = 27$) and for all *Ficus* species found in the literature ($n_A = 56$, $n_B = 72$). The boxes' upper and lower sides indicate the first and third quartiles, and the horizontal lines 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

Figure 2-3. 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 fruits, grey branches represent type B fruits, and white branches represent ambiguous reconstruction. At the tip 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 grey 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, eight and four, respectively, occurred on branches with type A fruits. For branch support value using maximum likelihood bootstrap and Bayesian posterior probabilities see Fig. 1.

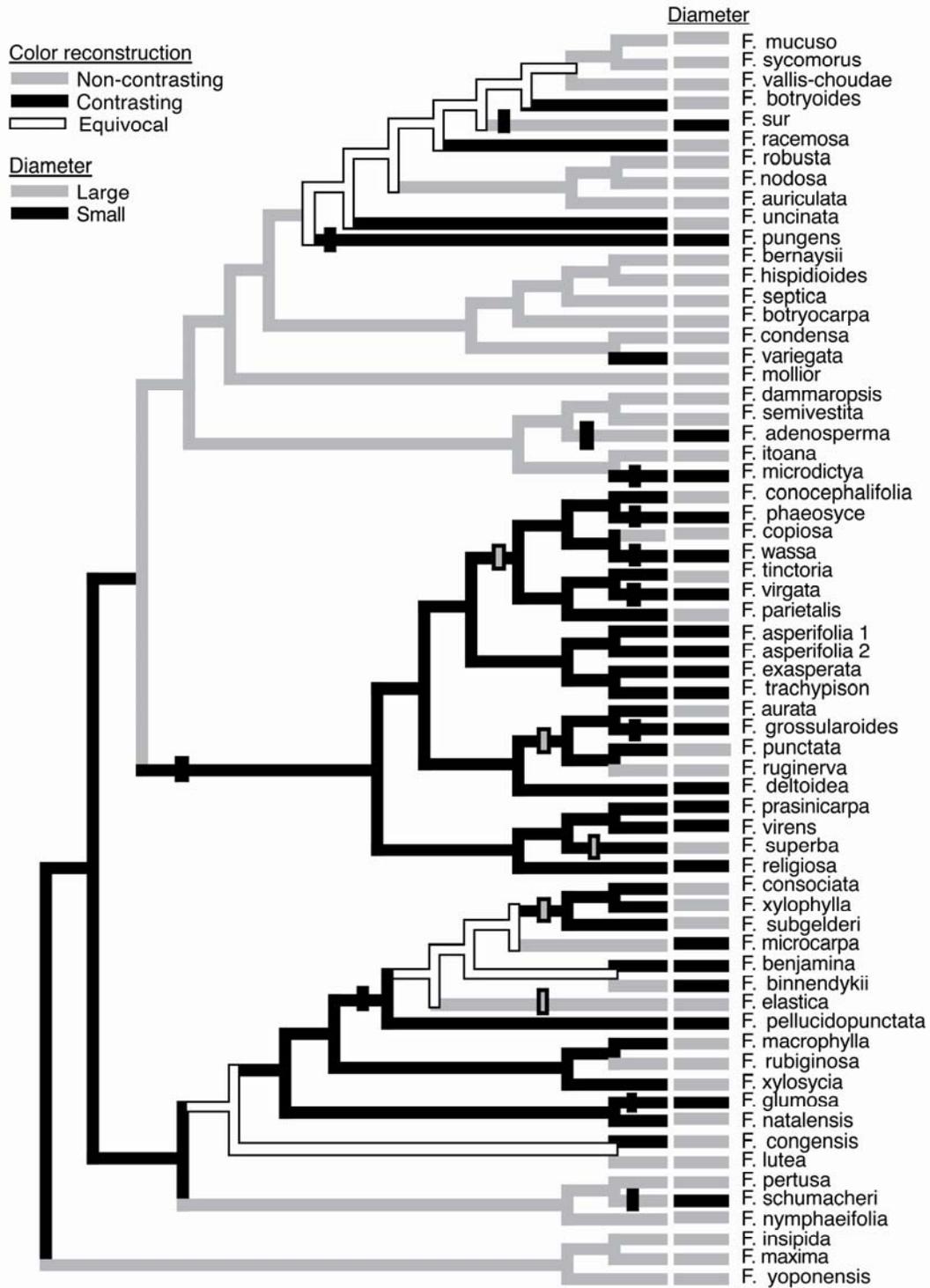


Table 2-1. Results from Maddison's Concentrated Changes tests showing all combinations of algorithms and character state reconstructions performed on the maximum likelihood tree using color and size as the independent variable (I and II, respectively).

		Non-conservative reconstruction				Conservative reconstruction			
Independent variable		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, which delays changes, or ACCTAN, which accelerates changes) Branches: number of gains and losses on the branches with the character state of interest. When considering color as the independent variable, Branches is the total gains and losses of "small size" that occur on branches with Type A (brightly colored) fruits. When considering size as the independent variable Branches is the total gains and losses of Type A colors on branches with small-sized fruits. MINSTATE and MAXSTATE are options for reconstructing character states at ambiguous nodes. MINSTATE uses the lowest trait value, whereas MAXSTATE uses the largest trait value.

Table 2-2. Data on fruit diameter, color, animal consumers, and distribution for the 64 species included in our phylogeny, as found in the literature. 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 10mm 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 were obtained mainly from Shanahan et al. (2001b), and secondarily from fieldwork by S. Lomáscolo. The superscript next to the distribution indicates the source from which this information was obtained.

<i>Ficus</i> species	Diameter (mm)	Color	Size code	Fruit Type	Avian consumers ^h	Mammalian Consumers ^h	Other consumers ^h	Distribution
<i>F. adenosperma</i>	10 ^a	Green ^a	Small	B	No data found	No data found		New Guinea ^b
<i>F. asperifolia</i>	10 ^a	Yellow or red ^a	Small	A or B	Pigeons	OW primates	Fish	Africa ^a
<i>F. aurata</i>	11 ^a	Orange ^a	Large	A	Passeriforms	Rodents		Borneo ^a
<i>F. auriculata</i>	60 ^g	Green ^g	Large	B		OW bats		China ^b
44 <i>F. benjamina</i>	8 ^a	Purple ^a	Small	A	Galliforms, Pisciforms, hornbills, Cuculiforms, parrots, pigeons, Passeriforms	NW and OW bats, OW primates, civet, rodents		Borneo ^a
<i>F. bernaysii</i>	12 ^f	Red ^f	Large	B	Cassowary, hornbills parrots, pigeon, Passeriforms	Marsupials, OW bats		New Guinea ^b
<i>F. binnendykii</i>	8 ^a	Red ^a	Small	B	Pisciforms, hornbills, pigeons, passeriforms	OW primates, rodents		Peninsular Malaysia ^a
<i>F. botryocarpa</i>	35 ^a	Green ^a	large	B		Marsupials		New Guinea ^b

Table 2-2. Continued

<i>F. botryoides</i>	29 ^e	Red ^e	large	A	No data found	No data found	Madagascar ^b
<i>F. condensata</i>	20 ^a	Green ^a	Large	B		OW bats	Borneo ^a
<i>F. congensis</i>	25 ^g	Red ^g	Large	A	No data found	No data found	Africa ^a
<i>F. conocephalifolia</i>	28.5 ^g	Red ^g	Large	A		OW bats	New Guinea ^b
<i>F. consociata</i>	12 ^a	Red ^a	Large	A	Pisciforms, hornbills, Cuculiforms, pigeons, Passeriforms	Tree shrews, Asiatic Black bear, OW primates, rodents	Peninsular Malaysia ^a
<i>F. copiosa</i>	29 ^a	Yellow ^a	Large	B	Cassowary, hornbills, Fig parrot	Marsupials, OW bats	New Guinea ^b
<i>F. dammaropsis</i>	60.5 ^g	Green ^g	Large	B	Honey eater	Marsupials, OW bats	New Guinea ^b
<i>F. deltoidea</i>	9 ^a	Red ^a	Small	A		OW primates	Borneo ^a
<i>F. elastica</i>	≥10 ^g	Green ^g	Large	B	Pigeons, Passeriforms	OW primates	New Guinea ^f
<i>F. exasperata</i>	9 ^a	Red ^a	Small	A	Hornbills, Turaco	OW bats, OW primates, Artiodactyla	Africa ^a
<i>F. glumosa</i>	9 ^a	Red ^a	Small	A	Pisciforms, pigeons, Passeriforms	OW primates	Africa ^a
<i>F. grossularoides</i>	10 ^d	Red ^d	Small	A	Passeriforms		Borneo ^b
<i>F. hispidioides</i>	35 ^a	Green ^a	Large	B		OW bats	New Guinea ^b
<i>F. insipida</i>	40 ^a	Green ^a	Large	B		NW primates, NW bats, Procyonids, tapirs	Turtle, fish Venezuela ^b
<i>F. itoana</i>	34 ^a	Green ^a	Large	B		OW bats	New Guinea ^b

Table 2-2. Continued

<i>F. lutea</i>	15 ^a	Yellow or red ^a	Large	A or B	Hornbills, pigeons, Passeriforms	OW bats, OW primates, elephants	Africa ^a
<i>F. macrophylla</i>	17.5 ^g	Purple ^g	Large	A	Cuculiforms, parrots, pigeons, Passeriforms	OW bats	Australia ^c
<i>F. maxima</i>	25 ^a	Green ^a	Large	B		NW bats, NW primates	Brazil ^b
<i>F. microcarpa</i>	<10 ^g	Purple ^g	Small	B	Pisciforms, hornbills, Cuculiforms, parrots, pigeons, Passeriforms	Marsupials, OW bats, OW primates	New Guinea ^b
<i>F. microdictya</i>	10 ^a	Red ^a	Small	A	No data found	No data found	New Guinea ^b
<i>F. mollior</i>	16 ^a	Green ^a	Large	B	No data found	No data found	Australia ^c
<i>F. mucuso</i>	40 ^g	Orange ^g	Large	B	Pigeons	OW bats, OW primates, elephants	Africa ^a
<i>F. natalensis</i>	13 ^g	Red ^g	Large	A	Pisciforms, hornbills, Turacos, pigeons, Passeriforms	OW bats, OW primates, Artiodactyla, elephants	Africa ^a
<i>F. nodosa</i>	28 ^a	Green ^a	Large	B	Hornbills	Marsupials, OW bats	New Guinea ^b
<i>F. nymphaeifolia</i>	22 ^a	Green or red ^a	Large	A or B		NW bats	South America ^a
<i>F. parietalis</i>	15 ^a	Red ^a	Large	A	Pisciforms, hornbills, Passeriforms	OW primates, rodents	Brunei ^c

Table 2-2. Continued

<i>F. pellucidopunctata</i>	9 ^a	Purple ^a	Small	A	Pisciforms, hornbills, Trogoniforms, pigeons, Passeriforms		Borneo ^b
<i>F. pertusa</i>	14 ^a	Yellow or red ^a	Large	A or B	Guans, Pisciforms, Coraciforms, Trogoniforms, parrots, Passeriforms	Marsupials, NW bats, NW primates, Procyonids	Venezuela ^b
<i>F. phaeosyce</i>	9 ^g	Red ^g	Small	A	Cassowary, hornbills, pigeons, Passeriforms	Marsupials, OW bats	New Guinea ^b
<i>F. prasinicarpa</i>	7 ^a	Red ^a	Small	A	Parrots		Solomon Islands ^b
<i>F. punctata</i>	49 ^a	Red ^a	Large	A		OW primates, rodents	Borneo ^b
<i>F. pungens</i>	7 ^g	Red ^g	Small	A	Hornbills, parrots, pigeons, Passeriforms	Marsupials, OW bats	New Guinea ^b
<i>F. racemosa</i>	25 ^c	Green or red ^c	Large	A or B	Galliforms, Pisciforms, hornbills, Cuculiforms, parrots, pigeons, Passeriforms	OW bats, OW primates, bears, civet, Artiodactyla, rodents	Australia ^b

Table 2-2. Continued

<i>F. religiosa</i>	10 ^g	Purple ^g	Small	A	Pisciforms, hornbills, Cuculiforms, pigeons, Passeriforms	OW bats, OW primates, Artiodactyla	Lebanon ^c
<i>F. robusta</i>	75 ^g	Green ^g	Large	B	No data found	No data found	New Guinea ^b
<i>F. rubiginosa</i>	12.5 ^g	Green ^g	Large	B	Cuculiforms, parrots, pigeons, Passeriforms	OW bats	Australia ^c
<i>F. ruginerva</i>	37.5 ^d	Orange ^d	Large	B	Hornbills	OW primates, rodents	Borneo ^b
<i>F. schumacheri</i>	5 ^a	Green ^a	Small	B	No data found	No data found	French Guyana ^c
<i>F. semivestita</i>	33 ^f	Green ^f	Large	B		OW bats	New Guinea ^b
<i>F. septica</i>	30 ^a	Green ^a	Large	B	Coraciiform, pigeons, Passeriform	Marsupials, OW bats, OW primates, rodents	New Guinea ^b
<i>F. subgelderi</i>	12 ^d	Orange ^d	Large	A	Pisciforms, hornbills, parrots, pigeons, Passeriforms	Tree shrew, OW primates, rodents	Brunei ^c
<i>F. superba</i>	20 ^g	Purple ^g	Large	A	Galliforms, Pisciforms, hornbills, Cuculiforms, parrots, pigeons, Passeriforms	OW bats, OW primates, civet, rodents	Borneo ^b

Table 2-2. Continued

<i>F. sur</i>	19 ^a	Red ^a	Small	B	Pisciforms, hornbills, Turacos, pigeons, Passeriforms	OW bats, OW primates, Artiodactyla	Africa ^a
<i>F. sycomorus</i>	26 ^a	Yellow ^a	Large	B	Pisciforms, hornbills, Turacos, pidgeons, Passeriforms	OW bats, OW primates, civet, hyrax	Africa ^a
<i>F. tinctoria</i>	14 ^a	Red ^a	Large	A	Hornbills, parrots, pigeons, Passeriforms	Marsupials, OW bats, OW primates, rodents	New Guinea ^b
<i>F. trachypison</i>	7 ^a	Red ^a	Small	A	Hornbills, parrots, pigeons, Passeriforms	Marsupials, OW bats	New Guinea ^b
<i>F. uncinata</i>	22 ^a	Red ^a	Large	A		Rodents	Brunei ^c
<i>F. vallis-choudae</i>	33 ^a	Yellow or red ^a	Large	B	Pigeons	OW bats, OW primates	Africa ^a
<i>F. variegata</i>	27 ^a	Green or red ^a	Large	A or B	Cassowary, hornbill, parrots, Passeriforms	Marsupials, OW bast, OW primates, rodents	Australia ^b
<i>F. virens</i>	8 ^a	Yellow ^a	Small	A	Cassowary, Pisciforms, hornbills, Cuculiforms, parrots, pigeons, Passeriforms, Cuculiform, pigeons, Passeriforms	OW bats, OW primates, carnivores, rodents	New Guinea ^b
<i>F. virgata</i>	8 ^a	Orange ^a	Small	A	pigeons, Passeriforms	OW primates	New Guinea ^b

Table 2-2. Continued

<i>F. wassa</i>	10 ^a	Red ^a	Small	A	Cassowary, hornbill, parrots, pigeons, Passeriforms	Marsupials, OW bats	New Guinea ^b
<i>F. xylophylla</i>	25 ^a	Orange ^a	Large	A	Pigeons, Passeriforms		Brunei ^c
<i>F. xylosyca</i>	13 ^a	Red ^a	Large	A	Pigeons, Passeriforms		New Guinea ^b
<i>F. yoponensis</i>	18 ^a	Green ^a	Large	B	Passeriforms	NW bats, NW primates, Procyonids, Artiodactyla, rodents	Panama ^a

Superscripts denote the source from which the information was obtained: a. Dominy et al. (2003); b. Weiblen (2000); c. Jousellin et al. (2003) ; d. Laman and Weiblen (1998) ; e. Missouri Botanical Garden website, at <http://mobot.mobot.org/W3T/Search/vast.html>; f. George Weiblen, pers. comm.; g. Pers. obs.; h. Shanahan (2000)
NW stands of New World, and OW stands for Old World.

CHAPTER 3 SIGNAL CONVERGENCE IN FRUITS: A RESULT OF SELECTION BY FRUGIVORES?

Introduction

To overcome the constraints imposed by immobility, many plants depend on animals for dispersal of pollen and seeds. Animal pollinators and dispersers cue on different plant signals to find flowers and ripe fruits. Yet the great diversity of plant signals is poorly understood -- it has been variously attributed to chance, to environmental factors, and to the sensory ecology of mutualistic animals (Ben-Tal and King 1997; Gumbert et al. 1999; Johnson and Steiner 2000). The latter, in particular, constitutes a long-debated central question in the field of plant-animal interactions: Do animal mutualists shape the evolution of plants, causing plant signals to converge into pollination and seed dispersal syndromes (Janson 1983; Johnson and Steiner 2000; Ridley 1930; van der Pijl 1961; 1969)? Syndromes are sets of flower or fruit traits hypothesized to evolve under the selective pressure of pollinators and seed dispersers, respectively. Because many unrelated plants are visited by similar mutualists, and because visitation rate is commonly density-dependent, an adaptive explanation for signal convergence is that plants sharing the same suites of visitors might benefit from converging on a similar phenotype to attract mutualists. This “Signal Convergence hypothesis” is the main focus of this study.

Nevertheless, the general convergence of fruit or flower traits to syndromes that are reliably associated with (i.e., presumably signal to) specific animal vectors has been questioned. Detractors of the syndrome hypothesis argue that plants tend to benefit from different types of animal mutualists and, conversely, plant-visiting animals do not tend to specialize on only one type of plant (Herrera 1985; Johnson and Steiner 2000; Waser et al. 1996). This argument may be particularly relevant to the evolution of seed dispersal syndromes because plants show a lower degree of specialization towards seed dispersers than they do towards pollinators (Bluthgen et al.

2007). From an adaptive viewpoint, the low degree of specialization in seed dispersal will result in disparate selective pressures on fruit appearance and will thus reduce the likelihood of signal convergence. Some authors claim that apparently non-random combinations of fruit traits are evidence of syndromes (Gautier-Hion et al. 1985; Janson 1983; Kalko et al. 1996; Knight and Siegfried 1983; Korine et al. 2000; Willson et al. 1989). However, when those combinations of traits have been scrutinized in phylogenetically controlled studies, the syndromes are generally not supported (Fischer and Chapman 1993; Herrera 1987). This difference in approach and opinion has generated much controversy.

We argue that one reason studies addressing the role of frugivores in shaping fruit evolution have reached disparate conclusions is that fruit color, one of the most important fruit traits reflecting differential food selection by distinct frugivore assemblages (Voigt et al. 2004), has been measured inappropriately. Color as a visual signal might be particularly important in defining syndromes because signals represent the first step in the interactions between plants and animals. Natural selection should favor signals (fruit colors) that elicit a response in the receiver (the frugivore) that increases the fitness of the sender (the plant) (Endler 1992). From this, we can derive that the efficiency of a signal not only depends on the signal's form (i.e., the spectral reflectance of the fruit), but also on the receiver's perception (i.e., the frugivore's neural circuitry to interpret the color signal) (Endler 1992). Nonetheless, evolutionary studies on fruits and frugivores have typically reported color qualitatively and according to human visual perception (Fischer and Chapman 1993; Gautier-Hion et al. 1985; Janson 1983; Lord et al. 2002), or they have ignored color completely (Jordano 1995), probably because of the difficulty of quantifying color.

Another important property of signals is their conspicuousness, which is quantified as the signal-to-noise ratio, and in the case of visual signals is directly correlated with the contrast between the color of the object and that of the surrounding environment. Natural selection should maximize conspicuousness to the receiver. In the case of fruits, the contrast between the color of the fruit and the foliage background should be greatest to the disperser to which the signal is targeted. Although fruit conspicuousness has been shown to be important for fruit detection (Burns and Dalen 2002; Regan et al. 2001; Schmidt et al. 2004a; Sumner and Mollon 2000a), the contrast between the color of the fruit and that of its background has never been incorporated in a study of dispersal syndromes.

Our study focuses on the role of fruit color as a signal to two of the most common frugivorous taxa, primates and birds. Under the Signal Convergence hypothesis, we test whether fruit color can be used as a signal to seed dispersers. We predict that:

1. Primates and birds should be able to distinguish visually between fruits that are primarily dispersed by one of them and those that are primarily dispersed by other group;
2. Fruit colors should be more conspicuous to the disperser (primates or birds) to which they are signaling.
3. The color of fruits dispersed mainly by primates should converge to colors that are different from those dispersed mainly by birds.

One assumption of our study is that primates and birds can have differential effects on plant fitness according to their differences in seed treatment and recruitment requirements of different plants. Although support is scant, some published studies suggest that this is a fair assumption (Clark et al. 2001; Poulsen et al. 2002).

To test prediction 1 of the Signal Convergence hypothesis we use vision models to analyze fruit signals according to the color vision capabilities of the main types of frugivores, primates and birds (Osorio and Vorobyev 1996; Regan et al. 2001; Vorobyev and Osorio 1998). These vision models approximate visual capability of specific frugivores based on their cone sensitivity (see Materials and Methods). To test prediction 2, we define conspicuousness as the contrast in color between the fruit and the foliage background (Endler 1992) and use vision models to approximate conspicuousness of fruits to each frugivore type. To test prediction 3, we compare the fruit colors of bird and primate dispersed plants belonging to the same family and genus.

To test these predictions, we use the largest spectral data set of fruit colors analyzed to this date. For 406 plant species with known fruit consumers, we demonstrate that primates are physiologically capable of discriminating between fruits dispersed by them and those dispersed by birds based solely on color. Likewise, we show that birds can do the same and that this pattern transcends geographic areas, and persists even when phylogenetic effects are controlled. We also discuss why fruit colors were not always more conspicuous to the targeted disperser.

Materials and Methods

Fruit Color and Disperser Data

Data on reflectance spectra of fruits and fruit consumers for each plant species were obtained mainly from the literature and partly from our fieldwork. A total of 406 species were included, of which 177 were primate-dispersed species from Guyana (111), Uganda (46), and Venezuela (20), and 229 were bird-dispersed species from Florida (29), Europe (99), Venezuela (31), and other unspecified regions (70) (Regan et al. 2001; Schaefer et al. 2007; Schmidt et al. 2004a; Sumner and Mollon 2000b). In all cases, color was quantified using a portable spectrometer and a special light source that reflects equally in all parts of the spectrum. Fruit reflectance was obtained as the proportion of reflectance by a white standard. The spectrometer,

light, and white standard varied among studies but were consistent within each study. Although the use of different equipment adds an uncontrolled source of variation in the data, we considered that, if the predicted patterns were found in reflectance of primate versus bird dispersed fruits, this would be *despite* the lack of consistence in color measuring devices, and not *because of* it.

Spectra were averaged in 5 nm intervals from 400-700 nm. Unfortunately, data on fruit reflectance under 400 nm (the UV range) were not available for primate-dispersed fruits, so we left this part of the visual range of birds out of our analyses. Our results are therefore conservative because differences between primate and bird perception of fruit color are likely more pronounced if UV vision is accounted for.

Vision Model

Because color is interpreted by the brain based on the input of light to different types of cones cells with different sensitivity to specific wavelengths (Bowmaker and Hunt 2006; Regan et al. 2001), we calculated the light input to the different cone types for each frugivore group. Our vision model combines the reflectance spectrum of each fruit with the color vision ability for each frugivore (represented by the peak sensitivity of the cone cells for each frugivore) and yields three variables per species of fruit, corresponding to the input to the brain by the short, medium, and long wavelength cones of the frugivore (Fig. 3-1). For primates, we used cone peak sensitivities corresponding to humans, (440, 540, and 570 nm), which are very similar to those of some other trichromatic primates (e.g., *Alouatta* spp.) (Bowmaker and Hunt 2006; Regan et al. 2001). For birds, we used cone peak sensitivities for the Blue Tit (*Cyanistes caeruleus*, 372, 456, 544, and 609 nm). We calculated the relative cone excitation values, called quantum catches (Fig. 3-1), which represent the basic sensory input for color vision (Kelber et al. 2003). Although cone peak sensitivity varies within each of these groups of frugivores (Bowmaker et al.

1997; Bowmaker and Hunt 2006), these differences are small relative to differences between birds and primates.

All cone excitation values were transformed to the natural logarithm to achieve normality and were analyzed with Discriminant Function analysis (DFA) to test whether bird-dispersed fruits and primate-dispersed fruits were significantly discriminated using the quantum catch of bird and primate cones. Discriminant Function Analysis finds the combination of the variables that best discriminates between the groups, and allows us to identify each variable's contribution to the discrimination. These variable combinations are called Linear Discriminant Functions (LDF). If birds can visually discriminate between fruits commonly dispersed by them and those commonly dispersed by primates, a significant discrimination should be obtained using the variables yielded by our vision model for birds and, likewise, for primates.

Data Analysis

Prediction 1: Color discrimination by birds and primates: To evaluate the accuracy of the DFA and its predictive power, we split our data set of 406 species into two parts and derived the LDFs with one part (species from Guyana, Florida, and unspecified geographic regions), called the “training” DFA, and tested the predictive value of LDFs on the other part of our data, a completely independent data set (species from Uganda, Europe, and Venezuela), called the validation DFA. This is a powerful test of the validity of the discrimination between primate and bird fruits by primate and bird vision, because the pattern of discrimination will remain significant only if it is consistent across very different biogeographical regions, such as the Paleotropics, Paleo-temperate areas, and the Neotropics. We also tested the predictive value of the linear discriminant functions by classifying the cases as “bird” or “primate” fruits based on their score on the LDF derived with the “training” DFA. The higher the percentage of species that were well classified (known bird-dispersed species correctly classified as a bird-dispersed

species), the higher the predictive value is of the linear discriminant functions. This was done for the “training” and the validation data sets.

Prediction 2: Conspicuousness of the fruits to frugivores: To test whether primate-dispersed fruits were more contrasting against the foliage than bird-dispersed fruits from a primate’s visual perspective, we first calculated the chromatic contrasts of 86 primate fruits and 188 bird fruits against the foliage background as the Euclidean distance between the color of a fruit and the color of the structure against which it is viewed by the frugivore. Spectra were normalized to the same brightness by dividing the reflectance at each wavelength by the total reflectance for each species. This provides contrasts due to the color of the fruits and not to their brightness (Schmidt et al. 2004a), which can vary greatly with light availability. The resulting contrasts for primate and bird fruits were entered into the primate-vision model and the results were compared using a Mann-Whitney U test. To ensure that the differences between fruit types are actually due to differences in reflectance by the fruit itself and not by the background, which is beyond the scope of the Signal Convergence hypothesis, we also calculated fruit contrasts against the mean of the background for the 256 species included in the analysis and compared the results with another Mann-Whitney U test.

Prediction 3: Phylogenetic control: Closely related species are not independent data points because they share a common ancestor. We controlled for such phylogenetic effects by doing a pair wise comparison of the mean score on the linear discriminant function for bird and primate dispersed species within each family of plants. To do such a comparison, we ran another DFA with all the species together and then only for the 22 families that had both bird and primate dispersed species. We calculated the mean score for bird and primate dispersed species per family. Based on the results of the training and validation data sets, plants dispersed by birds

scored consistently higher on the LDF separating the groups, and therefore we tested whether the scores of bird-dispersed species were significantly higher than primate-dispersed species of the same families using a one-tailed Wilcoxon Signed Rank test. We performed the same test on the mean scores within 12 genera for which we had bird and primate dispersed species. If the difference between bird and primate-dispersed species within each family or genus was significant, we concluded that the pattern of discrimination observed was not driven by phylogeny.

Results

Prediction 1: Color Discrimination by Birds and Primates

The “training” DFA resulted in significant discrimination between bird- and primate-dispersed fruits when using bird cone sensitivity peaks (Wilk’s $\lambda = 0.680$, numerator d.f. = 3, denominator d.f. = 205, $p < 0.00001$) and primate cone sensitivity peaks (Wilk’s $\lambda = 0.649$, numerator d.f. = 3, denominator d.f. = 205, $p < 0.00001$) (Fig. 3-2). Classification accuracy was fairly high, with 82% of 111 primate-dispersed species classified correctly and 76% of 98 bird-dispersed fruits classified correctly. Given the high negative loading of the medium wavelength variable (representing green light that primarily stimulates the medium-wavelength cone) and the high positive loading of the short wavelength variable (representing blue light) (Table 3-1), our results suggest that primate-dispersed fruits tend to be greener than bird-dispersed fruits, which tend to be more blue or purple.

The validation DFA also showed significant discrimination between bird and primate-dispersed fruits for bird (Wilk’s $\lambda = 0.638$, numerator d.f. = 3, denominator d.f. = 192, $p < 0.00001$) and primate cone sensitivities (Wilk’s $\lambda = 0.616$, numerator d.f. = 3, denominator d.f. = 192, $p < 0.00001$) (Fig. 3-2). Classification accuracy was also high, with 80% of 66 primate-

dispersed species being classified correctly and 82% of 130 bird-dispersed fruits classified correctly.

Prediction 2: Conspicuousness of the Fruits to Frugivores

Contrary to the predictions under the Signal Convergence hypothesis, primate-dispersed fruits are less conspicuous (i.e., contrast less against the foliage) to primates than are bird-dispersed fruits (Mann-Whitney $U = 4,273$, $p < 0.001$, $n = 256$). Unfortunately, we could not test conspicuousness of bird and primate-dispersed fruits to birds because UV reflectance is required to obtain an accurate measure of conspicuousness to birds. We are confident that the difference in contrasts is due to differences in fruit color and not background color because the contrasts calculated with the mean reflectance of the background for all 256 species included was also significantly different from a primate's perspective (Primates: Mann-Whitney $U = 8,584$, $p < 0.001$, $n = 256$).

Prediction 3: Phylogenetic Control

Our phylogenetic test comparing the mean scores on the linear discriminant functions of bird and primate-dispersed fruits within each family using a Wilcoxon Signed Rank test showed that bird-dispersed fruits tended to score higher on the LDF than did primate-dispersed fruits, although the difference was not as large as that found at the genus level (Family level: one-tailed test, $V = 77$, $p = 0.056$ for both bird cone peak sensitivity, and $V = 77$, $p = 0.056$ for both primate cone peak sensitivity; Genus level: one-tailed test, $V = 10$, $p = 0.021$ for bird cone peak sensitivity, and $V = 7$, $p = 0.005$ for primate cone peak sensitivity).

Discussion

Our results generally support the first prediction of the Signal Convergence hypothesis, which states that frugivores should be able to discriminate between the color of fruits dispersed by them and those of fruits dispersed by other frugivores. Based on the model representing the

visual capabilities of primates and birds, we conclude that these frugivores can differentiate between fruits typically dispersed by primates and those by birds. This is important because color could not be considered a signal to frugivores if frugivores were not capable of distinguishing colors.

Even though the discrimination is significant, a considerable amount of overlap exists between bird- and primate-dispersed fruits. This result is expected because, although a frugivore may more often consume fruits that are more conspicuous to them than those that are more cryptic, they will not feed exclusively on fruits with conspicuous colors. Frugivores can also find fruits based on senses other than vision, and they can feed on fruits found by chance from a nearby fruiting tree. Plants with exclusive seed dispersers are exceptional, and neither the Signal Convergence, nor the Dispersal Syndrome hypotheses imply exclusiveness of frugivores. If syndromes are an evolutionary response to seed dispersers, they should result from the response of a plant species to selection by those seed dispersers that improve its fitness, even though many other not-so-efficient frugivores may still feed on the same plant.

If we could include other cues used by these frugivores to find fruits in our model, we would likely obtain sharper discrimination. The fact that, as shown by our study, the color of some bird-dispersed fruits fall well within the range of a primate's visual perception, does not mean that bird fruits are necessarily conspicuous to primates and that they can easily find them. Primates may need a combination of cues to make the fruits conspicuous and fully available to them, such as color, odor, shape, texture, and/or location on the tree (Dominy 2004; Dominy et al. 2001). Our study is limited to testing only one trait (color), which we use to define dispersal syndromes and, although we believe color is one of the most important cue used by diurnal frugivores to find fruits, we acknowledge that it is certainly not the only one.

It is surprising that the quantum catch on the long wavelength cone, representing red light vision, was unimportant in discriminating between bird- and primate-dispersed fruits. Red fruits are commonly associated with bird dispersal more than with primate dispersal (Janson 1983; Wheelwright and Janson 1985; Willson and Whelan 1990), but so are black fruits. Red and black reflectance spectra differ dramatically in the amount of long wavelength light reflected and hence, bird dispersed fruits result in highly variable quantum catch for the cone responsible of long wavelength absorbance. Hence, reflectance at long wavelengths is not a consistent discriminant because the within group variance is too high. What red and black fruits do have in common is their high contrast against the foliage, which may be why birds are often associated with fruits of those colors (Schmidt et al. 2004a).

Our second prediction, that the fruits dispersed by a frugivore should be more conspicuous to that frugivore than fruits not dispersed by them, was not supported by our data. Primate fruits seem to be less conspicuous to primates than bird fruits. We propose several alternative explanations for the lack of congruence of the results for primates and birds, and the lack of complete support to our hypothesis, and we divide them into “adaptive” and “non-adaptive” explanation from the perspective of plant-frugivore communication. The adaptive explanations are consistent with the Signal Convergence hypothesis. First, it is possible that, while birds have such well developed color vision and may be primarily visual foragers, primates use other signals besides color, such as odor, size, and texture of fruits to assess their edibility (Dominy 2004; Laska et al. 2007). This may therefore derive in a stronger selective pressure on conspicuousness by birds than by primates. If this is the case, we predict that a similar test on conspicuousness to birds, bird-dispersed fruits should be more conspicuous to birds than primate-dispersed fruits. Also, by including fruits from varied geographical regions from the

New and Old World, we included a wide range of primates that have very different color vision capabilities (Bowmaker and Hunt 2006; Regan et al. 2001; SurrIDGE et al. 2003). In order to answer the specific question of whether the fruit/background color combination is optimized for dispersal by primates, we should differentiate between dichromatic and trichromatic primates, and we should also take into account the polymorphisms found among platyrrhine (i.e., New World) monkeys. If primates exert a strong selective pressure to maximize the signal-to-noise ratio in the fruits they eat, the signal will most likely be seen when studying specific groups of primates with well defined and similar color vision capabilities. This may not be as crucial when testing conspicuousness to birds because birds have a much higher within-guild similarity in cone sensitivities among very different taxa (Hart 2001), and therefore their potential selective pressure on fruit color may be similar among different bird species. We mentioned in the Introduction that variation in color vision among disperser types is greater than that within disperser type. However, within group variability, even if small, may affect the results of the test for fruit contrasts more than that of color discrimination.

Second, it is also possible that our measure of conspicuousness is simplistic. Sumner and Mollon (2000b) argue that, at equal contrast between background and fruit color, larger fruits are more conspicuous to primates simply because they are larger. That is, large-fruited plants may not be under as strong selective pressure as small-fruited plants to maximize the difference between background and fruit color. Under this hypothesis we predict that, among the primate-dispersed fruits, species with lower score on LDF 1 will be larger than species with higher LDF 1 score, because LDF 1 is negatively associated with reflectance of green light, which is less conspicuous against the foliage. This is similar to what Sumner and Mollon (2000b) showed when they correlated fruit size and reflectance at longer wavelength. However, his negative

correlation can also be explained by the effect of smaller, more visual frugivores, such as birds feeding on smaller fruits, as predicted by the Dispersal Syndrome hypothesis, rather than the sole effect of selection on conspicuousness by one type of frugivore (Lomáscolo et al. 2008; Sumner and Mollon 2000b). Whatever the ultimate explanation for this correlation, we suggest that conspicuousness to frugivores should be measured as a function of fruit size, in addition to the contrast against the background.

The non-adaptive explanations consider roles of color that are independent of plant-frugivore communication. Willson and Whelan (1990) identify 13 non-exclusive hypotheses that may explain fruit color patterns, of which six are completely unrelated to seed dispersers and include defense against pathogens or seed predators, as well as physiological, or phylogenetic constraints. Although we will not attempt a thorough discussion of all Willson and Whelan's (1990) hypotheses, we want to generally address some that seem particularly relevant to our study. First, the anthocyanins responsible for red and black colors in fruits have been shown to reduce fungal growth (Schaefer and Schaefer 2007) and, thus, attraction of seed dispersers by anthocyanines may be a by-product of the antimicrobial properties of these pigments. Red is also inconspicuous to certain insects that eat fruit but do not disperse seeds, so this pigment may have evolved to *hide* fruits from predators -- not to make them more conspicuous to seed dispersers (Willson and Whelan 1990). In this case, color should be considered an exaptation (Gould and Vrba 1982) instead of an adaptation to endozoochory.

Second, fruit pigmentation has been hypothesized to decrease some physiological constraints, such as thermoregulation. Because dark colors absorb more heat than light colors, it has been hypothesized that dark fruits have higher metabolic rates and therefore can reach maturity faster, reducing exposure to fruit predators. However, dark colors would also cool

faster as the radiation emitted by a body must equal the radiation absorbed (Kirchhoff's Law of Thermal Radiation), so dark fruits should be at a disadvantage in many tropical rain forests where clouds cover the sun light long periods of time. Yet dark-colored fruits are extremely common in these cloudy environments (Wheelwright and Janson 1985; Willson et al. 1989; Willson and Whelan 1990). The thermoregulation hypothesis does not explain the advantage of dark-colored fruits in the shaded understory either, where they are also very common (pers. obs.) Lastly, as Wheelwright and Janson (1985) point out, dark pigments are only deposited at the end of the ripening period and, hence, may contribute very little to the development of the fruit.

Third, fruit colors have also been hypothesized to be related to the plant's metabolic constraints. 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). This is especially relevant to large-fruited species: 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), which does not explain the importance of green fruits in environments of varying light levels, such as a rain forest or the forest understory.

Last but not least are phylogenetic constraints. Fruit traits in general have been shown to be highly conserved (Jordano 1995), probably due to the long generation time of plants in comparison with seed dispersers (Herrera 1985). Our comparison between species in the same genus suggests that similar colors among species dispersed by primates and those dispersed by birds are the result of convergence rather than shared common ancestry, at least in the 12 genera

included. Although not statistically significant, the trend found in 22 families also suggests the independent evolution of fruit color.

We emphasize the importance of measuring color objectively, using newly available portable spectrometers (Altshuler 2001; Altshuler 2003) and to interpret these colors based on the visual capabilities of the frugivores under study (Chittka and Raine 2006). Although primates have sometimes been merged with birds in a bird-primate syndrome because no differences in color were found between fruits dispersed by these two groups of frugivores (Gautier-Hion et al. 1985), we show that, while primates and birds overlap in the color of the fruits they consume, primates disperse more green fruits than do birds, and that birds disperse more blue fruits than do primates. These results match those of earlier studies in fruit choice by frugivores (Janson 1983; Wheelwright and Janson 1985), even when those studies classified color from a human perspective. We believe that this match, despite such different methods of measuring color, is because we are testing frugivores whose color vision capability is similar to that of humans. This is, of course, with the non-trivial exception of UV perception in birds, which we were unable to consider due to lack of UV reflectance information in the literature. We emphasize that using vision models may be especially important if the visual system of the frugivores under study (e.g., a dichromatic primate) differs significantly from our visual system. In the case of this study, if fruit reflectance in the UV range were included in our analysis, the discrimination between primate and bird-dispersed fruits using all four of a bird's cone peak sensitivity would almost certainly be sharper than that shown here. The results from the primate's perspective should not change because we would be incorporating reflectance information on a part of the spectrum that primates cannot see.

Although we cannot be sure that our model exactly represents how birds and primates perceive color, as color vision entails much more than cone excitation, our model provides an objective approximation of color vision by these frugivores. This is the first study that addresses the Signal Convergence hypothesis in a fruit-frugivore system, using such an objective measure of color and such a large data set of reflectance spectra (the largest analyzed to date).

Color is an important mediator in plant-animal communication (Chittka and Raine 2006; Johnson and Steiner 2000; Schaefer et al. 2004, this study) and we believe that learning the signaling role of color will shed light on understanding how animals and plants affect each other's fitness and evolution. This can only be done by using vision models that incorporate the receptive and cognitive processes of the receivers, which adds much needed objectivity in the assessment of how plant signal to animals (Schaefer et al. 2004).

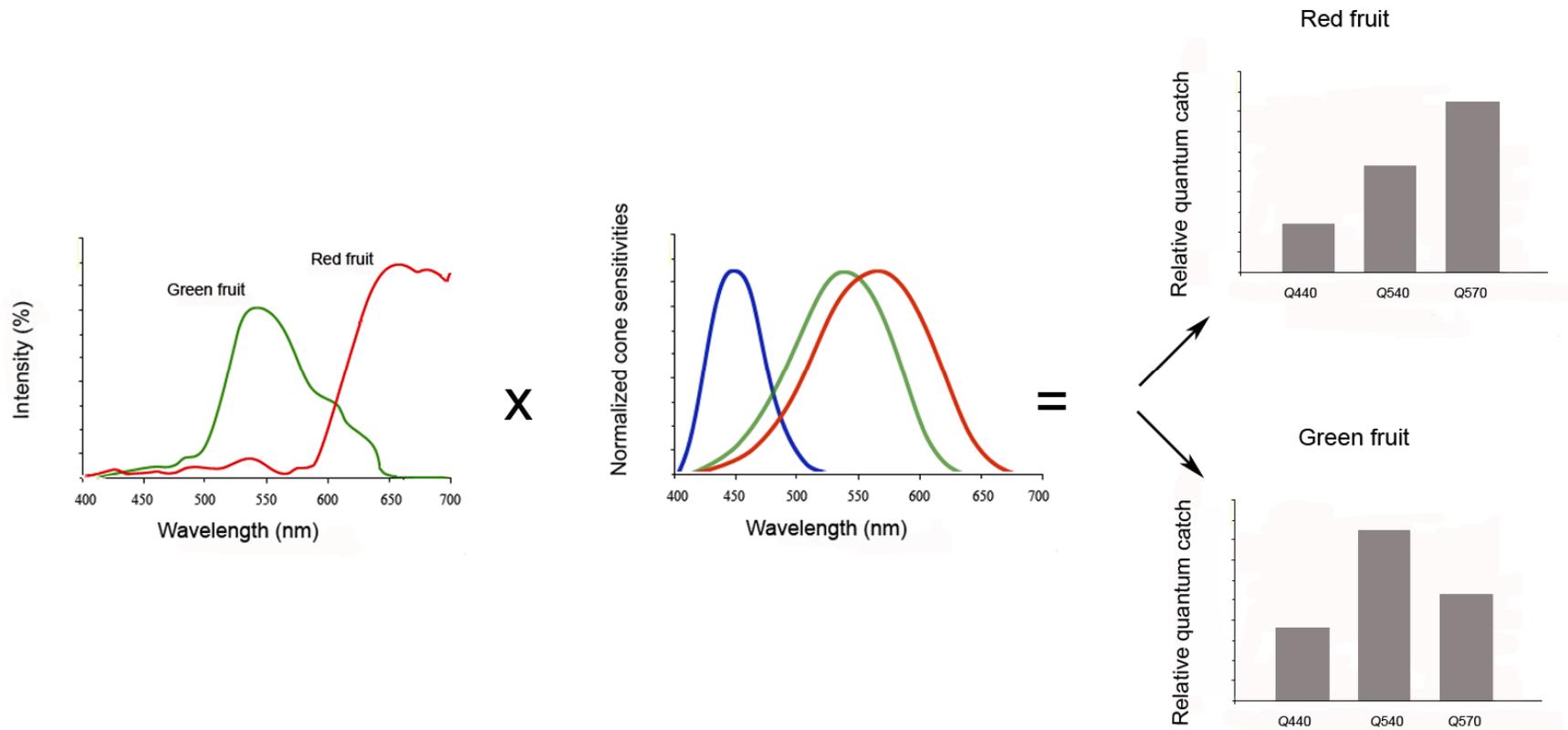


Figure 3-1. Reflectance spectra of two different fruits (left) combined with the peak cone sensitivities of a frugivore (middle) yields the quantum catch for the three cone types for each fruit (right). The quantum catch values approximate the frugivore's perception of color.

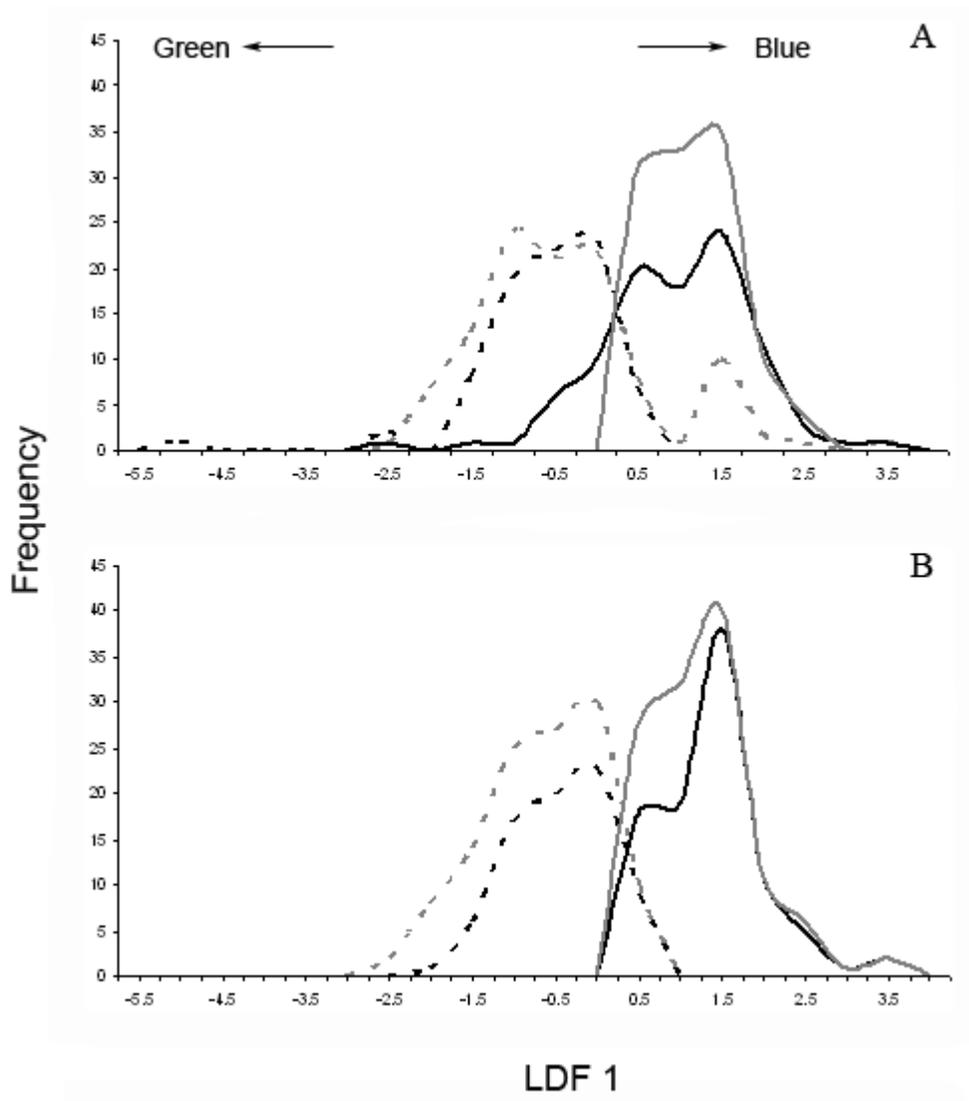


Figure 3-2. Results of the Discriminant Function analysis (DFA) based on (A) primate cone excitation and (B) bird cone excitation. Black lines correspond to the “training” DFA and grey lines to the validation DFA. Dashed lines represent primate-dispersed fruits and solid lines represent bird-dispersed fruits. Primate-dispersed fruits tend to have negative scores on the linear discriminant function, because they are greener than bird-dispersed fruits, which have more of a blue hue than primate-dispersed fruits

Table 3-1. Loadings of the quantum catch variables for primate and bird cone sensitivities for the “training” DFA

Variable	Loading on LDF 1	
	Primate vision	Bird vision
Short (Blue light)	1.396	1.234
Medium (Green light)	-2.758	-1.797
Long (Red light)	0.771	-0.071
Proportion of variation	1.00	1.00

CHAPTER 4
REVISITING THE DISPERSAL SYNDROME HYPOTHESIS: A PHYLOGENETIC
COMPARATIVE STUDY OF FRUIT TRAITS IN *FICUS* (MORACEAE)

Introduction

Seed dispersal by vertebrates occurs in tropical, temperate, xeric, and moist environments alike, both on land and in aquatic habitats (Adams et al. 2007; Godinez-Alvarez et al. 2002; Herrera 1987; Korine et al. 2000; Shanahan et al. 2001b; Spiegel and Nathan 2007; Traveset et al. 2004). Its selective advantage in various geological scenarios and historical periods is evidenced by its independent evolution in many plant lineages, from seed ferns, to gymnosperms and angiosperms (Herrera 1989; 2002b). It is particularly ubiquitous in moist tropical environments, where over 90% of woody species are dispersed by animals (Herrera 2002b), and where it has been proposed as one of the most important proximate mechanisms explaining high tree diversity (Janzen 1970; Terborgh et al. 2002). Yet, the question “How important are seed dispersers to fruiting plants?” may elicit fundamentally different answers from the ecologists and the evolutionary biologists in the field. While ecologists argue convincingly that seed dispersal is important to the fitness of fruiting plants, many evolutionary biologists argue just as strongly that there is little evidence seed dispersal “matters” to plants (i.e., that it has influenced the evolution of fruit traits).

Among the most cited lines of evidence for the contemporary role of seed dispersal in plant and animal communities are that it links the last phase of a plant’s reproductive cycle to the first phase of population recruitment (Herrera et al. 1994; Nathan and Muller-Landau 2000; Rey and Alcantara 2000; Schupp and Fuentes 1995), contributes disproportionately to forest recovery after natural disasters (Hjerpe et al. 2001; Thornton et al. 1996; Whittaker and Jones 1994), and dramatically reduces density-dependent mortality near the parent plant (Fragoso et al. 2003; Howe 1986; Howe and Miriti 2000; 2004; Howe et al. 1985; Schupp 1988). There is also

growing evidence that dispersal limitation (i.e., the inability for seeds to colonize sites appropriate for germination) may be a major process limiting plant recruitment, affecting plant spatial distributions and contributing to the maintenance of biodiversity (but see Clark et al. 2007; Clark et al. 1999; Hurtt and Pacala 1995; Muller-Landau et al. 2002; Murray and García-C 2002; Turnbull et al. 2000). Thus, plants species without dispersers may face extinction (Cardoso da Silva and Tabarelli 2000; Chapman and Onderdonk 1998; Cordeiro and Howe 2001; 2003). Conversely, reductions in fruit abundance may threaten frugivore populations (Moegenburg and Levey 2002; Redford 1992).

But evolutionary evidence is less clear-cut. Some argue that seed dispersal *is consequential* to the evolution of fruits and point to the apparently non-random combination of fruit traits (Cipollini et al. 2002; Gautier-Hion et al. 1985; Janson 1983; Korine et al. 2000), called “dispersal syndromes”, that match presumed preferences and fruit-handling abilities of different sets of frugivores. However, most evidence to date suggests that seed dispersal *is inconsequential* in shaping fruit traits, as factors other than seed dispersers can better explain fruit traits – these factors include phylogeny (Fischer and Chapman 1993; Herrera 1987; 1989; 1992; Jordano 1995), architectural constraints (Herrera 1987; 2002a), and growth form (Herrera 1987). Moreover, fruit traits presumably selected for by frugivore taxa such as birds or mammals are not always appealing to their putative dispersers (Tamboia et al. 1996), casting doubt on the validity of adaptive explanations for the association of those traits with particular sets of dispersers.

It is puzzling that researchers have failed to find support for the potential role of seed dispersers in the evolution of fruits because many assumptions that are central to the adaptive role of fruit traits hold true, namely: 1. seed dispersal is often important for plant reproduction

(Fragoso et al. 2003; Harms et al. 2000; Howe and Miriti 2000; 2004); 2. seed dispersers can exert disparate selective pressures on fruiting plants because disperser guilds treat seeds differently (Clark et al. 2001; Godinez-Alvarez et al. 2002; Poulsen et al. 2002), and may therefore affect plant fitness differentially; 3. disperser guilds perceive fruit traits differently and can differentiate fruit traits well (Hodgkison et al. 2007; Korine and Kalko 2005, Chapter 3, this dissertation), making it feasible for plants to use fruit traits to attract beneficial consumers and/or to deter detrimental consumers, and 4. some traits such as fruit size and color are not strongly phylogenetically conserved, suggesting potential for selection by seed dispersers (Jordano 1995; Lomáscolo et al. 2008 (Chapter 2, this Dissertation); Voigt et al. 2004).

We argue that one of the reasons for the contradictory conclusions of evolutionary studies is the analytical approach used by different studies. First, some studies have failed to consider the role of phylogeny in determining the similarity in fruit-trait combination among plant species (Janson 1983; Kalko et al. 1996; Korine et al. 2000), which fails to account for the fact that species may not be independent data points. Second, those who controlled for the non-independence of species have either focused at higher taxonomic levels or have used estimates of the phylogenetic relatedness of species (instead of explicit phylogenies), methods that often decrease the power to find a correlation between disperser type and variation in fruit traits, when it exists (Bremer and Eriksson 1992; Fischer and Chapman 1993; Jordano 1995). Third, some of the most influential studies come from temperate regions (Herrera 1987; 2002a) where fruit diversity is limited compared to tropical regions; therefore great variation in tropical fruit traits remains unexplained. Fourth, thorough studies that investigated the variation of fruit traits with dispersal mode have analyzed fruit traits separately (Herrera 2002a; Jordano 1995), whereas frugivores most likely assess whole fruits based on many variables simultaneously. Finally,

dispersal guilds have often been defined very broadly (i.e., birds vs. mammals, Janson 1983; Jordano 1995), which hinders the definition of the potential selective pressure of frugivores on the fruit traits of interest.

Perhaps the most glaring reason why evolutionary studies are not conclusive on whether frugivores have selected for fruit traits is that some of the most important fruit traits used by frugivores to find and select fruits have been excluded or mismeasured. The most striking examples of this are fruit color and odor. These traits are commonly used by plants to signal fruit ripeness (Sumner and Mollon 2000b) or nutritional composition (Catoni et al. 2008; Goff and Klee 2006; Schaefer et al. 2008; Schaefer and Schmidt 2004), and are two of the most important cues used by frugivores to find and select fruits (Kalko et al. 1996; Laska et al. 2007; Thies et al. 1998; Voigt et al. 2004; Wheelwright and Janson 1985; Willson and Whelan 1990). In particular, they are quantified from the perspective of human perception. This can generate significant and unintended biases, given that other animals sense the world differently than humans and that most plants use color and odor to signal to animals other than humans (Bennett et al. 1994; Schaefer et al. 2004). For example, most passerine birds can see UV light, while humans and most other mammals cannot (Bennett and Cuthill 1994; Bennett and Thery 2007; Hart 2001; Peichl 2005). Likewise, most mammals have a more developed sense of olfaction than humans and, especially, than birds (Clark 1991; Evans 2006; Heymann 2006; Wysocki and Preti 2004).

Our work improves on previous studies by 1. focusing at the species level and controlling for the non-independence of species using a molecular phylogeny; 2. studying tropical plant genus with highly variable fruit traits; 3. using a multivariate phylogenetic comparative approach to simultaneously test for the correlated evolution of seven fruit characteristics that are predicted

to be important for frugivores to find fruits, namely fruit size, color, odor, softness, contrast against the background, location on the tree, and exposure away from foliage; 4. focusing on two well defined disperser guilds with clearly different potential for selection on fruit traits; and 5. reducing human biases by using quantitative measures of color using a spectrometer, and of odor through gas chromatography/mass spectrometry.

We test two hypotheses for co-occurring sets of fruit traits. First, the Phylogenetic Hypothesis: fruit traits are inherited from ancestors – they are the result of shared phylogenetic history. Second, the Dispersal Syndrome Hypothesis: fruit syndromes are the result of selective pressure by sets of seed dispersing frugivores that differ in behavior and ecology. Our objective is to tease apart these hypotheses by determining how much variation in fruit traits is attributable to phylogeny and how much is attributable to selective pressure by frugivores. We focus on two well defined groups of frugivores, birds and bats, which are predicted to select for the following dispersal syndromes: **1. Passerine bird syndrome:** Because passerine birds have a relatively narrow gape, lack teeth, have acute color vision (tetrachromatic, including a peak in the UV range, Bowmaker et al. 1997; Das et al. 1999; Hart 2001), a poor sense of smell, and usually perch to pick fruits, fruits preferred by passerines should be small, visually conspicuous (hues of red, purple or black, contrasting against leaf or bark background), less odorous than bat-dispersed fruits, and presented among foliage along branches. **2. Bat syndrome:** Because bats have teeth (and therefore can manipulate fruits with their mouth and eat them piecemeal), are mostly nocturnal (which limits their perception of color), use chemical cues to find fruit (Bianconi et al. 2007; Hodgkison et al. 2007; Mikich et al. 2003), and grab fruits in flight or land on trees and crawl to reach fruits (Elangovan et al. 2001; Kalko et al. 1996), fruits preferred by them should be larger than bird-dispersed fruits, odorous, and less visually conspicuous (hues of

green, yellow, pale orange, or brown, non-contrasting against leaf or bark background). Also, they will grow along the trunk (caulicarpic) and, in general, they will be more exposed away from the foliage than fruits eaten by birds (Ridley 1930; van der Pijl 1969). We demonstrate that these two groups of frugivores have had an evolutionary impact on fruit traits within *Ficus*.

Methods

Study Site and General Specifications

Data were collected between September 2004 and December 2005 in a lowland forest near the village of Ohu (145° E, 5° S) in Madang Province, Papua New Guinea, (Fig. 4-1). Data on fruit color, color contrast against the background, odor, size, softness, and exposure to frugivores (location of fruits on branches or trunk, distance from closest leaves, and peduncle length) for each of 42 fig species were obtained by averaging records from between 3 and 8 individuals per species. For each individual tree, between one and 10 fruits were measured and averaged for all traits.

Study system: the natural history of figs

Ficus (Moraceae) provide a unique opportunity to study the evolution of fruit traits in relation to seed dispersers because its species are unusually variable in fruit color, size, presentation, and type of disperser. Fig fruits are technically closed infructescences, that are hollow and shaped like an urn, most probably selected to allow the characteristic internal pollination provided by wasps (Janzen 1979; Weiblen 2002). (For more information on fig pollination and its relation with the seed dispersal mutualism, see Chapter 5, this dissertation.) Fig infructescences are called “syconia”, a term we use interchangeably with “fruits” because they are functionally fruits; i.e., they are comprised of seeds and nutritious pulp, and they attract and reward seed dispersing animals. They can be red, purple, black, pink, orange, yellow, green, or brown. Syconia range in diameter from 3-80 mm and grow from the tips of branches, at the

base of leaves, on the trunk from ground level to the canopy, and even from the roots (Laman and Weiblen 1998, G. Weiblen pers. comm., S. Lomáscolo pers. obs.). In terms of life form, figs can be free standing, epiphytic, hemi-epiphytic, or “parasitic” stranglers of other trees. They are also geographically widespread (Shanahan et al. 2001b). More than 700 species are found worldwide in tropical forests, thereby regularly co-occurring with different sets of dispersers. Dispersers include bats, arboreal and terrestrial mammals, volant (passerines, hornbills, fruit doves, parrots, etc.) and flightless birds (e.g, Cassowaries), fishes, and reptiles (Shanahan et al. 2001b).

Figs are best known for their tight mutualism with pollinating wasps, in which one species of fig is typically pollinated by one species of wasp. The mechanism of pollination is quite similar in all species of *Ficus*: a gravid female wasp carrying with pollen enters the closed inflorescence, pollinates the flowers and lays eggs in some of the ovaria. Therefore, viable seeds develop from some flowers and wasp larvae develop from others (Janzen 1979). Because pollinators may influence flower anatomy (Temeles and Kress 2003) the fact that all fig species have virtually the same pollinator contributes to making *Ficus* a good study system: floral morphology is fairly constant and, therefore, most differences found in fruit characteristics among figs should be largely independent of floral traits.

In many forests and across continents, figs are considered a “keystone” resource because they produce large fruit crops that ripen during seasons when few other fruits are available (Lambert and Marshall 1991; Terborgh 1986). Figs may also be important to frugivore communities due to their high calcium content (O’Brien et al. 1998). On the flip side, frugivores appear important for figs because figs depend heavily on seed dispersal for regeneration (Titus et al. 1990).

Figs have often been deemed generalists because they produce large amounts of fruits with low nutritional quality, fruit year-round, and attract large numbers of dispersers from different guilds. These attributes have likely discouraged researchers from considering figs as a study system for detecting dispersal syndromes. However, when figs are studied with attention to species-specific differences, it becomes quickly apparent that many species are almost exclusively visited by specific guilds of frugivores, such as bats, arboreal mammals, and birds (Kalko et al. 1996; Korine et al. 2000; Shanahan 2000; Shanahan and Compton 2001; Shanahan et al. 2001a), and only some of them fruit continuously (S. Lomascolo, pers. obs.).

Quantification of Visits to Figs

All data on fig consumption come from figs that contain viable seeds; these figs are commonly called “seed” figs (see Chapter 5). We recorded consumption of figs using two video cameras (Sony DCR-HC40), which were set up 3-5m away from the fruiting *Ficus* tree and visited only to change tapes or batteries. To record both diurnal and nocturnal frugivores, we started videotaping at 06:00 and ended at 10:30, and continued again between 18:30 until 23:00. We recorded nocturnal visits using an infrared light (Sony HVL-IRH2). We aimed one camera at the ground to record terrestrial frugivores and the other either at a branch or at the trunk, depending on where most of the ripe figs were located. We recorded between one and four individuals of 29 species of figs for a total of 1019 hours. Unless a frugivore was seen not to eat any fruit, all frugivores that entered the tree were considered seed dispersers. For some species for which fruit data were collected but no dispersers were recorded, we obtained frugivore information from Shanahan et al. (2001b). One species for which no frugivore was recorded, *F. botryocarpa* had been repeatedly pointed out by villagers as a commonly bat-dispersed species, so we also included it in the analysis as a bat-dispersed species.

Fruit Traits

Fruit color: We quantified fruit color using a USB2000 portable spectrometer (Ocean Optics, Inc) and a PX-2 pulsed xenon light source, which took reliable readings between 300 and 740nm. This spectrum includes the range of wavelengths visible by humans, plus the ultraviolet 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 fruits constant we fastened a non-UV filtering microscope slide to the opening of the hole where the sensor was introduced. To obtain an average reflectance spectrum per fruit, we scanned each fruit three times in three different spots on the fruit. We calculated reflectance as the *proportion* of a Spectralon white reflectance standard (Labsphere, North Sutton, NH) and, hence, reflectance measures do not have units. The three spectra for each species were averaged every 5nm.

We obtained a quantitative measure of fruit color for each species using a Principal Components Analysis (PCA), which summarized the information for the complete spectrum by finding linear combinations of the original variables -- in this case, reflectance intensity every 5 nm (Clotfelter et al. 2007; Cuervo and Shine 2007; de Ayala et al. 2007; Endler 1990). This technique reduces multivariate data to several axes that summarize the variance in, and covariance among, the original variables. A separate PCA was done to obtain color variables for the group of 42 species of figs for which fruit data were collected, and for the group of 19 species of figs for which exclusive dispersal by birds or bats was recorded. These two groups were used in different statistical analyses to answer separate questions (see below). In both cases the first principal component (PC) had high positive loadings for all wavelengths throughout the spectrum, and hence represents fruit brightness (Fig. 4-2), i.e., the total amount of light reflected

by the fruit. Therefore, light fruits had high scores on PC 1 and dark fruits had low scores. Principal components 2 and 3 represent different hues. Hue refers to the “everyday” meaning of color, summarizing differences in reflectance of different wavelengths. The second PC distinguishes reflectance in the red versus the UV-blue wavelengths because it had positive loadings for wavelengths from 300 to ~600nm, and negative loadings from 600 to 740nm. Therefore, UV-blue fruits had a high positive score on PC 2, whereas fruits reflecting in the red part of the spectrum had high negative scores. The third principal component distinguishes reflectance in the green versus red and UV-blue range because it had high positive loadings of wavelengths between 450nm and 640nm. Thus, green fruits had a high score on PC 3 and UV-blue and red (and, therefore, purple) fruits had a low score. These three PCs explained 91% of the variance in reflectance and all had eigenvalues greater than one, which means that each explained more variation than any single original variable alone. Although PCs 4 and 5 also had eigenvalues higher than one, they only explained 4 and 3% of the variation, respectively. Because reflectance in all parts of the spectrum of interest was summarized by PCs 1 to 3, we considered that additional PCs did not contribute sufficiently to the color description of each fig species. Hence, we use only scores on PCs 1 to 3 in subsequent analyses.

Color contrasts were calculated as the Euclidean distance between the color of a fruit and the color of the structure against which it was exposed to the frugivore, which consisted of either leaves or bark, depending on the species. Spectra were normalized to the same brightness by dividing the reflectance at each wavelength by the total reflectance for each species. This generates contrasts due to the color of the fruits not to their brightness. These contrasts are called “chromatic” contrasts (Schmidt et al. 2004a) and are calculated as

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

were Q_f is the color spectrum of the fruit and Q_b is the color spectrum of the background structure; λ is the wavelength in nm and the sum corresponds to the complete spectrum between 300-740nm.

Fruit odor: To capture volatile compounds, we collected fruits in the field, brought them to the lab, and placed them inside bags made of inert material (Reynolds oven baking bags). A vacuum pump (Welch, model #2522B-01) sucked air into the bag through a carbon filter on one end and forced it out of the bag through a filter at the opposite end filled with Super Q (Alltech), which adsorbed volatiles emitted by the fruits. For a given collection of fruits in a bag, we ran the pump for four continuous hours, which began no more than three hours after the fruits had been collected from the tree. To extract fruit volatiles from the Super Q filters, we flushed methylene chloride through the filters. We then used gas chromatography to separate and quantify the amount of different (unknown) volatiles. We also used mass spectrometry to identify some of the compounds. Because there were too many compounds ($n \sim 75$) to identify or analyze separately and because we were most interested in determining whether bird and mammal 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 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 that produced the volatile.

Fruit size, and softness: We measured fruit diameter with calipers to the nearest 0.5 mm and we used this as an estimate of overall fruit size. To measure fruit softness, we determined an arbitrary scale between 1 (hardest; fruit surface did not indent when pressed against fingers) to 4 (softer; fruit surface indented easily when pressure was applied to fruit between the fingers). To

achieve consistency in this arbitrary measure the same researcher recorded this measurement throughout the study. Fruit size and softness, together with fruit color and odor will be referred to as “appearance traits”, to differentiate them from traits reflecting exposure to the frugivores (“exposure traits”).

Fruit exposure traits: To quantify fruit position away from the foliage, we estimated the distance between the fruit and the nearest leaf to 1.0 cm for the highest and the lowest fruit in the tree, and averaged these values to incorporate the variation within each tree. Another exposure variable was dichotomous: fruits growing from branches versus those growing from the trunk. A third measure of exposure was peduncle length, the distance (± 0.5 mm) between the base of the fruit and the point where the peduncle attached to the branch or trunk.

Data Analysis

All statistical tests were done using R version 2.6.0 (R Development Core Team 2008), except for the Evolutionary Principal Components Analysis (Dyreson and Maddison 2006), for which we used Mesquite 2.01 (Maddison and Maddison 2007).

Identification of correlation in fruit traits: Principal Components Analysis (PCA) was used to identify the main axes of variation in the fruit data. In this case, PCA was used to identify the main differences between bird and bat-dispersed figs, and to define which variables covaried along the axis separating figs dispersed by these two guilds. Because variables were measured in different units and differed by up to three orders of magnitude, they were standardized to zero mean and unit standard deviation.

We performed two different PCAs, one on the 19 species of figs that were dispersed by either birds or bats (hereafter “Disperser PCA”), and one on the 42 species of figs for which we collected fruit data (hereafter “Fruit Trait PCA”). The Disperser PCA was done to objectively define fruit syndromes by identifying the suites of traits that differed between bird-dispersed and

bat-dispersed figs. Species with mixed dispersal were not included in this analysis because they were predicted to have intermediate characteristics between species exclusively dispersed by birds or by bats, and our goal was to identify the differences between bird and bat fruits. We tested whether the difference between bird and bat dispersed species on the PC axis explaining the most variation between the two groups was significant using a Mann-Whitney U test. The Fruit Trait PCA included all the fig species for which fruit trait data were collected, and it included species dispersed by birds, by bats, by both, and those for which disperser data was not available. The goal of the Fruit Trait PCA was to explore the overall pattern of variation in the fruit variables and to identify whether the trait correlation observed in the bird and bat-dispersed species could be generalized to a wider set of fig species. Also, we explored whether fig species with mixed dispersal had intermediate trait values between bird and bat fruits.

Phylogenetic control: To test whether the pattern of fruit trait variation was most likely explained by phylogeny or could be considered adaptive, we performed an Evolutionary Principal Components Analysis (EPCA) (Dyreson and Maddison 2006; Schlick-Steiner et al. 2006). This EPCA is based on evolutionary transitions (the difference in trait value between each species and the most recent ancestor, as reconstructed by a parsimony criterion, minimizing the sum of squared changes) instead of the raw variables used in a standard PCA. While standard PCA maximizes the variation in the traits among species, EPCA maximizes evolutionary change incurred by each species after they split from their ancestor, thus avoiding the problem of non-independence of species (Dyreson and Maddison 2006). Just as explained above for PCA, EPCA was completed on the 19 species set of bat and bird-dispersed species (Disperser EPCA) and on the 42 species set (Fruit Trait EPCA). To test whether there was a

significant difference between bird and bat dispersed species on the EPC 1 resulting from the Disperser EPCA we used a Mann-Whitney U test.

A second method of testing the role of phylogeny in explaining variation in fruit traits is to correlate that variation with genetic relatedness among species (Voigt et al. 2004). If what determines covariation of fruit traits (i.e., dispersal syndromes) is phylogenetic history, then closely related plant species should share combinations of fruit traits. If, instead, dispersal syndromes result from divergent selective pressures of different types of seed dispersers, similarity of fruits between any given pair of species should not be related to the genetic relatedness of those species. For each possible pair wise comparison of the 42 fig species for which we collected fruit data, we calculated genetic relatedness between all possible pairs of taxa, quantified in PAUP* (Swofford 2003) as the number of character changes along the tree between each pair of species corrected by the model of evolution chosen to build the phylogenetic tree. This genetic difference between two taxa is called “patristic distance” and is proportional to the sum of branch lengths between them. We also calculated the difference in fruit appearance between all possible pairs of taxa as the absolute value of the difference in the score on the PC 1 resulting from the Fruit Trait PCA, which was the most important in separating bird and bat-dispersed species. We then tested for a correlation between these estimates of genetic relatedness and difference in fruit appearance ($n = 861$ pairs) using a Mantel test (Mantel 1967). The Mantel test avoids the problem of non-independence of species by first calculating two distance matrices, one based on genetic distance and the other based on difference in trait appearance, and then randomly permuting rows and columns within each matrix. The probability of the correlation observed, as quantified by r^2 , is calculated based on the null distribution created by the randomization.

Volatile compounds in figs with different dispersers: A Discriminant Function Analysis based on presence/absence of 73 volatile compounds was used to identify the most important compounds distinguishing fig species dispersed primarily by birds (5 fig species), bat (13 species), bandicoot (2 species), and by more than one of these groups (10 species). To test the significance of the discrimination, we calculated a Kruskal-Wallis (K-W) statistic for the difference in the score on Linear Discriminant Function (LDF) 1, which mainly separated between the groups in the original data matrix, then randomized the matrix and re-calculated a K-W statistic for the random matrix. Column totals were fixed to control for common volatile compounds, while row totals were allowed to vary. This process was repeated 10,000 times to obtain a random distribution of the K-W statistic and then compared our observed K-W statistic to the random distribution.

Phylogenetic reconstruction

Although four phylogenies of the genus *Ficus* have been published (Jousselin et al. 2003; Lomáscolo et al. 2008; Rønsted et al. 2005; Weiblen 2000), none have all the species for which we collected fruit data. We therefore reconstructed our own phylogeny, using DNA sequences from GenBank and some from N. Rønsted (Department of Medicinal Chemistry, University of Copenhagen). We constructed one tree for the 19 species exclusively dispersed by birds and bats (necessary for the Disperser EPCA), and one for the 42 species for which we had data on fruit traits (necessary for the Fruit Trait EPCA) (Fig 4-3). A third tree that included 29 species with known dispersers (the 19 species exclusively dispersed by birds and bats, and 10 species with mixed dispersal) was constructed in order to map dispersal mode (Fig. 4-4). We used a maximum likelihood criterion, which searches for the phylogenetic tree that best fits the data, given a particular model of evolution. The model of evolution that best described our data was determined using the Akaike Information Criterion from the model set examined by

MODELTEST 3.6 (Posada and Crandall 1998). We conducted a heuristic search in PAUP* with 10 random sequence additions using the appropriate model (GTR+G for all three data sets) and parameters recommended by MODELTEST 3.6 (Posada and Crandall 1998). We assessed support on the phylogeny using 500 maximum likelihood bootstrap replicates using GARLI 0.951 (Zwickl 2006). Unresolved nodes were resolved for further comparative analyses based on published phylogenies. In the few cases where the species were not included in any published phylogeny, we resolved it randomly. Several random combinations were tested and all yielded similar results, so all results reported are based on one resolution, as seen in Figure 4-3.

Results

Visits to Fig Trees

Out of the 29 fig species that were videotaped, we obtained disperser data for 15. Data on frugivore consumption of 13 additional species were obtained from Shanahan et al (2001b), and one from the knowledge of local villagers. Most species were dispersed by bats (14 species), birds (5 species), or both (10 species). Two species were recorded as exclusively bandicoot dispersed and they were included in the exploratory analysis of volatile compounds in bat, bird, bandicoot and bird dispersed fruits. Two observations were too few to justify the addition of a Bandicoot Syndrome, so we did not include it in other analyses.

Based on a parsimony reconstruction of dispersal mode we identify at least three switches from bat to bird dispersal and from bat to mixed dispersal (Figure 4-4). Although many sister species share the same dispersal mode, this reconstruction suggest dispersal mode has changed independently many times in this group of figs.

Disperser PCA and EPCA

The first principal component (hereafter PC) explained 38% of the variation in the data, and is associated, in decreasing order of magnitude, with contrast against the background (-),

softness (-), distance from leaves (+), growth from branches (-), peduncule length (+), size (+), and brightness (+) (Fig. 4-5A, Table 4-1). Bird-dispersed species tend to have lower scores on PC 1 than bat-dispersed species, suggesting that dispersed species are more contrasting against the background, softer, more often growing among the foliage and on branches, have shorter peduncules and are smaller than bat-dispersed species (Fig. 4-5A). All of these characteristics match those predicted for bird and bat syndrome fruits. The second PC explained 17% of the variation and it seemed to mainly separate two fig species (*F. semivestita* and *F. edelfeltii*) from the rest. We saw no identifiable biological relevance of this separation with respect to disperser type, and therefore we will not report this PC further. Controlling for phylogeny, the EPCA reveals a similar pattern (Fig. 4-5B), which is confirmed by a highly significant correlation between the scores of each fig species on PC 1 and EPC 1 ($r^2 = 0.77$, d.f. = 17, p-value < 0.0001). The loadings for contrast against the background, softness, distance to closest leaf, peduncule length, size, and did not change substantially between PC 1 and EPC 1, although their order of importance changed slightly (Table 4-1). The fruit traits that showed most difference between PC 1 and EPC 1 were fruit brightness and odor, which became more important in distinguishing between bird and bat figs when phylogenetic effects were controlled and location, and location of fruits on branches or trunk, which became less important (Table 4-1). Although the loading of the color variable defining UV-blue versus yellow (PC3) is not high, it is worth noting that its sign changed from positive in PC 1 to negative in EPC 1.

Fruit Trait PCA and EPCA

The Fruit Trait PCA with 42 fig species shows a pattern of ordination similar to the Disperser PCA. The first PC explained 38% of the variation in fruit data and was associated, in decreasing order of magnitude, with contrast against the background (-), growth from branches (-), peduncule length (+), distance from leaves (+), softness (-), size (+), and odor (+) (Fig. 4-6A,

Table 4-2). Except for contrast against the background, color variables were not as important to explain the variation among fig species. The EPCA reveals a similar pattern to that of PCA, but the ordination is not as clearly matched to the PC axes, as it is in the Disperser EPCA. Fig species seem to be separated on EPC 2 in a similar fashion to their spread on PC 1 (Fig. 4-6). Because EPC 1 and EPC 2 explain a very similar amount of variation (21.4% vs 20.3%, respectively) and defining which is the main axis of variation is unimportant, and because bird and bat dispersed species are separated in both dimensions, we report both axes in detail. A strong, positive correlation ($r^2 = 0.79$, d.f. = 40, p-value < 0.0001) between the score of each species on PC 1 and on EPC 2 suggests that the ordination of species does not change substantially after controlling for phylogeny. EPC 2 shows higher loadings of exposure variables, as it is associated, in order of magnitude with distance from leaves (-), growth from the trunk (-), and peduncle length (-), and also with contrast against the background (+) and odor (-), whereas EPC 1 is largely based on differences in fruit appearance, as it is associated, in order of magnitude, with brightness (+), size (+), and softness (-), and also with peduncle length (+)(Table 4-2). Most exposure variables have similar loadings on EPC 2 to those of PC 1, but the relative importance is slightly different. In particular, fruit size and two of the color variables defining fruit hue became less important in EPC 2. On the other hand, the loadings of most appearance variables on EPC 1 are similar to those on PC 1.

The correlation between genetic relatedness, as measured by branch length between pairs of species, and the difference in the score on the PC1 axis is significant but explains less than 3% of the variation in the data ($r^2 = 0.023$, d.f. = 859, p-value < 0.02) (Fig. 4-7).

Volatile Compounds

The DFA on volatile compounds resulted in significant discrimination between bird, bat, bandicoot, and mixed dispersal modes (Fig. 4-8A). The twelve most important compounds

associated with bat-dispersed species are shown in Table 3. The scores on LDF 1, which separated bird from bandicoot and bat dispersed figs, were compared with a Kruskal-Wallis test and are significantly different from each other (K-W $\chi^2 = 20.38$, d.f. = 3, p-value = 0.0001). The randomization of the data matrix and re-calculation of the Kruskal-Wallis statistic 10,000 times showed that the probability of obtaining by chance a discrimination as significant as that found with the original matrix was 0.0002. Figure 4-8B shows an example of the discrimination achieved with a random matrix.

Volatile compounds more strongly associated with bat dispersal were mostly esters (Pentanol acetate, Acetamide, Ethyl octanoate, and Hexenoic acid, and a small, unidentified compound), with some sesquiterpenes (Muurolene, Panasimsen, and Germacrene D) and an unsaturated alcohol (unidentified), which generally coincides with previous studies of volatiles in bat-dispersed figs (Hodgkison et al. 2007).

Discussion

Our phylogenetic comparative study of 10 variables describing fruit appearance and accessibility to frugivores suggests that fruit traits covary in a way predicted only under selective pressure by frugivores. We believe our results are particularly robust for two reasons. First, we controlled for phylogenetic effects using a species-level phylogeny. Although phylogenetic effects can be controlled in other ways (Fischer and Chapman 1993; Herrera 1987; Jordano 1995; Voigt et al. 2004), using a species-level phylogeny is currently the most accurate manner for estimating the true phylogenetic history of a given taxon. Second, we used a multivariate phylogenetic comparative analysis, which allowed us to search for patterns in many variables simultaneously without violating assumptions of independence. This approach provided an holistic analysis of the multiple traits that frugivores perceive and use to find and assess fruits. It is simplistic to assume that a frugivore assesses, for example, the size of a fruit independently of

its color, odor, or position in the tree. Just as the conspicuousness of any given fruit color is higher in large than small fruits (Sumner and Mollon 2000b), conspicuousness almost certainly depends on a combination of many traits that together make the fruit relatively easy or hard to find by a frugivore, based on its overall perception of the world.

Our results strongly suggest that bird and bat syndromes indeed exist in figs. In Papua New Guinea, figs in the Bird Syndrome are darker, more contrasting against the background, less odorous, less exposed away from the foliage, smaller and softer than figs in the Bat Syndrome. This list generally matches the set of traits previously suggested for Bird Syndrome and Bat Syndrome fruits (Knight and Siegfried 1983; Shanahan et al. 2001b). Our approach, however, allows us to conclude that this particular combination of traits reflects evolutionary transitions -- i.e., changes undergone by each fig species after splitting from an ancestral species. Therefore, these sets of traits are likely adaptive.

Birds and bats also showed an association with figs growing from branches or directly from the trunk, respectively, but the importance of these variables was dramatically reduced in the EPCA, which may suggest that the location of figs on a tree is most strongly determined phylogenetically. Although several alternative hypotheses have been proposed to explain the colors of fruits (Willson and Whelan 1990), or the correlated evolution between, for example, fruit color and size (Cipollini and Levey 1991; Lomáscolo et al. 2008 (Chapter 2, this Dissertation)), we are not aware of any alternative hypothesis that can explain the correlated evolution of fruit size, brightness, contrast against the background, odor, softness, and exposure away from the foliage. The most parsimonious explanation for the correlated evolution of these six traits is natural selection by seed dispersers.

The same general pattern is apparent in a much larger data set of fig species, although data on consumers are not available for all species. This suggests that Dispersal Syndromes exist and are adaptive, at least for the set of 42 fig species included in this study. Our results generate testable predictions about potential consumers and seed dispersers of fig species, especially those falling in the extremes of the ordination shown in Figure 4-6. Our results also show that frugivores overlap in the fruits they eat. Many fig species that fall close to the “bird” extreme of the dispersal syndrome axis (EPC 2 in the Fruit Trait EPCA) were recorded to be also (e.g., *F. microcarpa*, *F. phaeocyse*, *F. pungens*, *F. wassa*,) or even exclusively (e.g., *F. subulata*, *F. virgata*) dispersed by bats. These “exceptions” do not undermine our argument of the importance of frugivore selective pressure because the Dispersal Syndrome hypothesis does not assume exclusiveness of frugivores. The assumption is, instead, that the strongest selective pressure will come from the overall most efficient (sensu Schupp 1993) disperser. Because our disperser data came from tree visits recorded in the field or reported in the literature, we are likely considering only the most common disperser(s) of each fig species. Therefore, an important assumption behind our analysis is that the most common seed disperser is the most efficient seed disperser. Admittedly, this is an oversimplified view – many processes important to plant fitness occur after a frugivore consumes a fruit. We argue that the pattern we observed holds *despite* this generalization, not *because* of it. A better test of the Dispersal Syndrome hypothesis would consider dispersers that directly increase the fitness of the dispersed plant (i.e., increase seedling survival). We believe that if those data were used in a test similar to the one we have presented here, the Dispersal Syndromes we described here would be even more pronounced.

Another test of phylogeny's influence on co-variation in fruit traits was the correlation between genetic relatedness and fruit appearance (i.e., scores on PC 1 derived from the Fruit Trait PCA). Although closely related species tended to be more similar in appearance than non-closely related species, less than 3% of the overall variation in fruit trait combination is explained by genetic relatedness. Closely related species will always be more similar to each other than to random species because, by definition, they shared a much longer history. What is striking to us is that 97% of the similarity between figs must be explained by factors other than phylogenetic relatedness. This lack of correlation can also be visually appreciated in Figure 4-3, where the PC 1 score is mapped onto the phylogeny. Our results support the idea that fruit appearance is more labile than it had been previously argued (Bremer and Eriksson 1992; Herrera 1987; Jordano 1995) and, at least in figs, an important part of this variation appears to be explained by differences among dispersers. We want to note that our estimates of the amount of variation in fig traits that can be accounted for by frugivores may be conservative because EPCA and the correlation between genetic relatedness with difference in trait characteristics they take into account what is not shared by closely related species. As it has been previously noted by Westoby and colleagues (1995) and also by Harvey and Pagel (1991), trait variation that is inherited by common ancestry and is, thus, shared by closely related species, may also be adaptive. This is because closely related species might tend to converge on similar adaptations because they respond similarly to similar selective pressures because of their shared genes, and because they may tend to colonize similar habitats and, hence, be subject to similar selective pressures. Moreover, a characteristic that is shared by all members of the same clade might have been fixed because of its high adaptive value. Many phylogenetic comparative methods give priority to phylogeny over ecology to explain shared characteristics, but because we show results

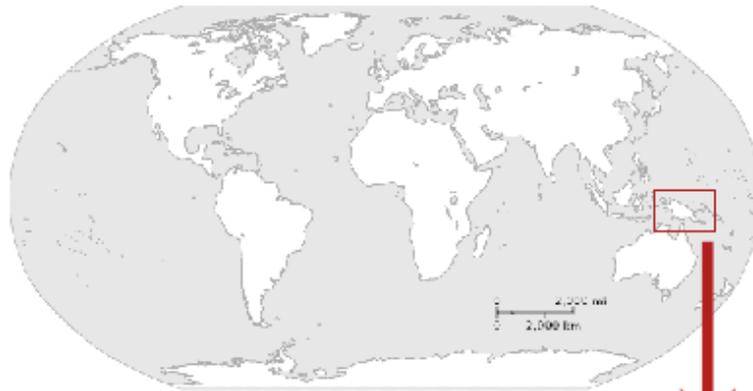
from non-phylogenetic and phylogenetic comparisons, we allow the reader to draw their own conclusions on the extent to which shared characteristics can be explained by either process

We suggest that Dispersal Syndromes should be thought of in terms of traits that make a particular species of fruit more *easy to find* by frugivores, and not more *attractive* to them. Although a seemingly subtle semantic distinction, we argue that distinguishing among traits that make a given fruit more or less *attractive* to a frugivore may lead to confusion and to false expectations under the Dispersal Syndrome hypothesis. Imagine, for example, a frugivore given a choice between fruits with different characteristics. Based on previous experience with other fruits and the post-ingestional benefits particular to those fruits (e.g., nutritional and health-related benefits, (Catoni et al. 2008; Goff and Klee 2006; Schaefer et al. 2008)), it may choose the one that is more *attractive* in those terms. However, a more natural scenario is one in which frugivores are not presented with choices but are instead searching for food to the best of their ability, given the limitations imposed by their physiology, anatomy, and foraging behavior. If a frugivore, while searching for, or feeding on a preferred fruit with high nutritional quality, encounters by chance fruits that are easily accessible, it will most probably consume those fruits too, even if those fruits are otherwise not *attractive*, just because other fruits may be scarce. In this way, birds may consume bat-syndrome fruits, and vice versa. The more *attractive* fruit may be preferred over the less attractive by the frugivore in a feeding trial during which the frugivore must choose between the fruits. But whenever fruits are relatively uncommon, such preferences likely disappear – *if found* both species will be equally consumed in nature. Instead, if a plant highly benefits from the dispersal of its seeds by a specific type of frugivore, the selective pressure on the plant will be to be *found* more readily by such a frugivore. With this distinction in mind, the expectation of frugivore exclusiveness may be unrealistic.

It is worth noting that fruit color per se, as described by hue, was relatively unimportant in discriminating between bird and bat fruits. However, two components of fruit color – fruit brightness and contrast against the background – were important in distinguishing figs dispersed by birds and bats. This result echoes results of previous studies that emphasize the role of contrast for bird-dispersed fruits (Schaefer et al. 2006; Schmidt et al. 2004a). Note, however, that in our dataset hue co-varied with brightness and may therefore still play a role (albeit a weak one) in defining Disperser Syndromes. Fig species with green and yellow fruits tended to have higher scores on PC 1 and EPC 1, and red and purple fruits tended to have low scores on these axes, which were highly positively associated with brightness. Chromatic contrasts also tended to be associated with color, as red and black fruits have higher contrast against green foliage (Schmidt et al. 2004a). This suggests that these other dimensions of color are more directly associated with bat or bird dispersal than reflected in our analysis. It would be interesting to repeat this analysis using other quantitative measures of color, such as hue and chroma (defined as the purity of the color, Endler 1990), to determine whether brightness and contrast still prevail over fruit hue in discriminating between bird and bat-dispersed fruits.

Although bat-dispersal seemed to be associated with specific volatile compounds, we did not test the evolutionary role of bats in selecting for those particular compounds. Because some of these compounds are also present in some fig species when fig inflorescences are receptive to pollinating wasps (Grison-Pige et al. 2002a; Grison-Pige et al. 2002b), it is possible that those compounds may have been selected for by fig pollinators, not dispersers. Hence, until more detailed studies on the evolution of these compounds are available, we suggest viewing these compounds as an exaptation (Gould and Vrba 1982), not an adaptation, to frugivore attraction.

Many fruit traits may be subject to selective pressure from multiple factors (Cipollini and Levey 1997; Mack 2000; Willson and Whelan 1990), but this does not undermine the importance of seed dispersers as a selective force that may generate and/or maintain fleshy fruit diversity. Any fruit trait may have several functions and therefore be subject to disparate selective pressures, generating complex patterns of trait covariation. Even so, traits with a strong influence on frugivore attraction will be subject to selection by frugivores. Although our data do not allow us to conclude that any particular trait of bird or bat-dispersed figs *originated* from the selective pressure of these seed dispersers (i.e., is an *adaptation* to seed dispersal), we believe that the correlated evolution of so many traits that were predicted *a priori* to play a role in frugivore attraction, strongly argues for an important role of seed dispersers on the evolution of fruits as a whole and, hence, in explaining the diversity of fleshy fruits.



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Figure 4-1. Map of Papua New Guinea in reference with a world map. Data were collected from areas surrounding the city of Madang, mainly from Ohu village (not shown).

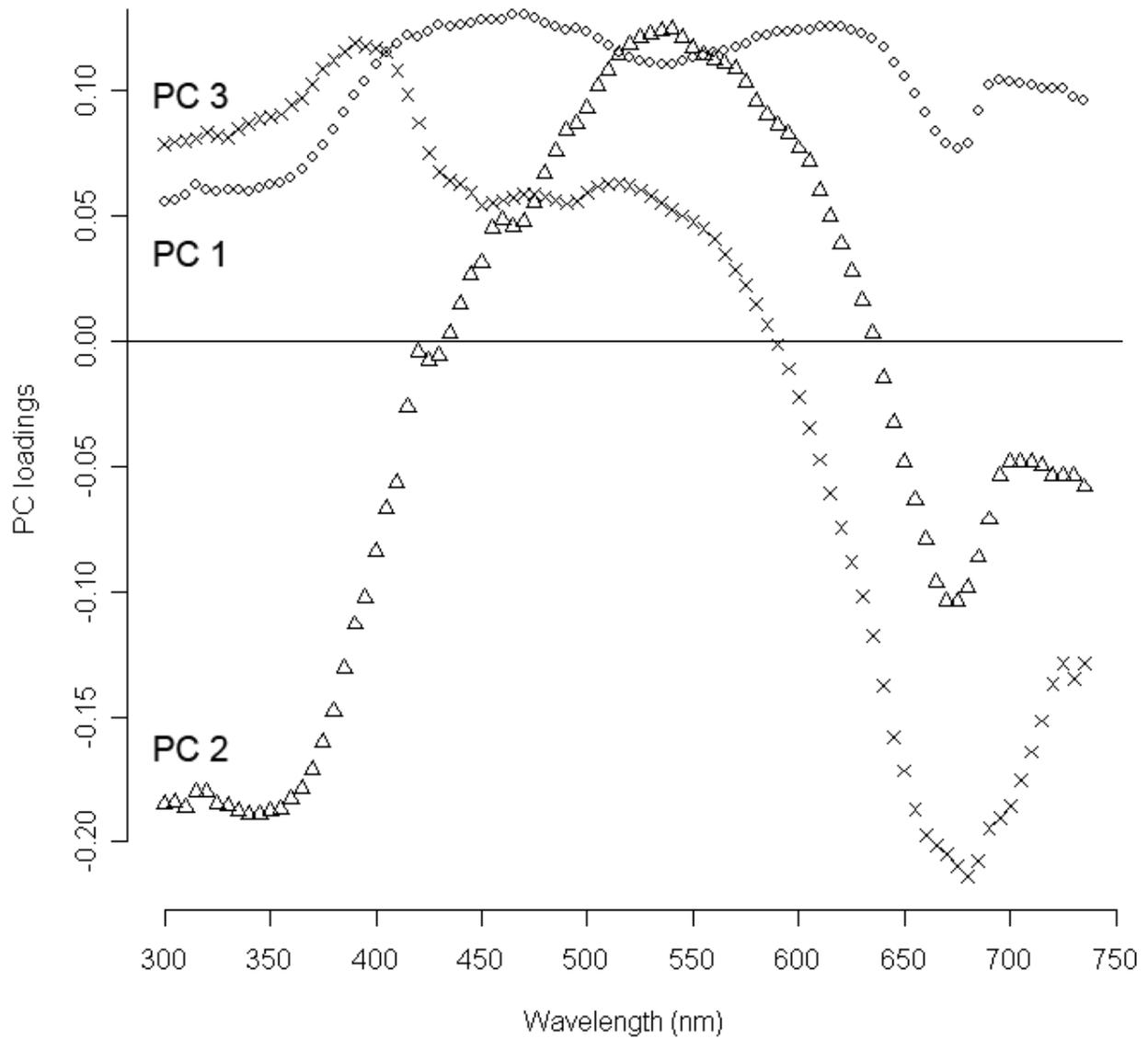


Figure 4-2. Loadings of the first three principal components (PC) on fruit reflectance spectra from 300 to 750nm, measured at 5nm intervals. PC 1 represents variation in mean brightness, PC 2 in green versus red coloration, and PC 3 in UV and blue coloration.

Figure 4-3. Maximum Likelihood tree of 42 fig species. Nodes with bootstrap support value ≥ 70 are marked with a star. The trait mapped in the phylogeny is the score of each species on PC 1, the dispersal syndrome axis, using a maximum parsimony criterion.

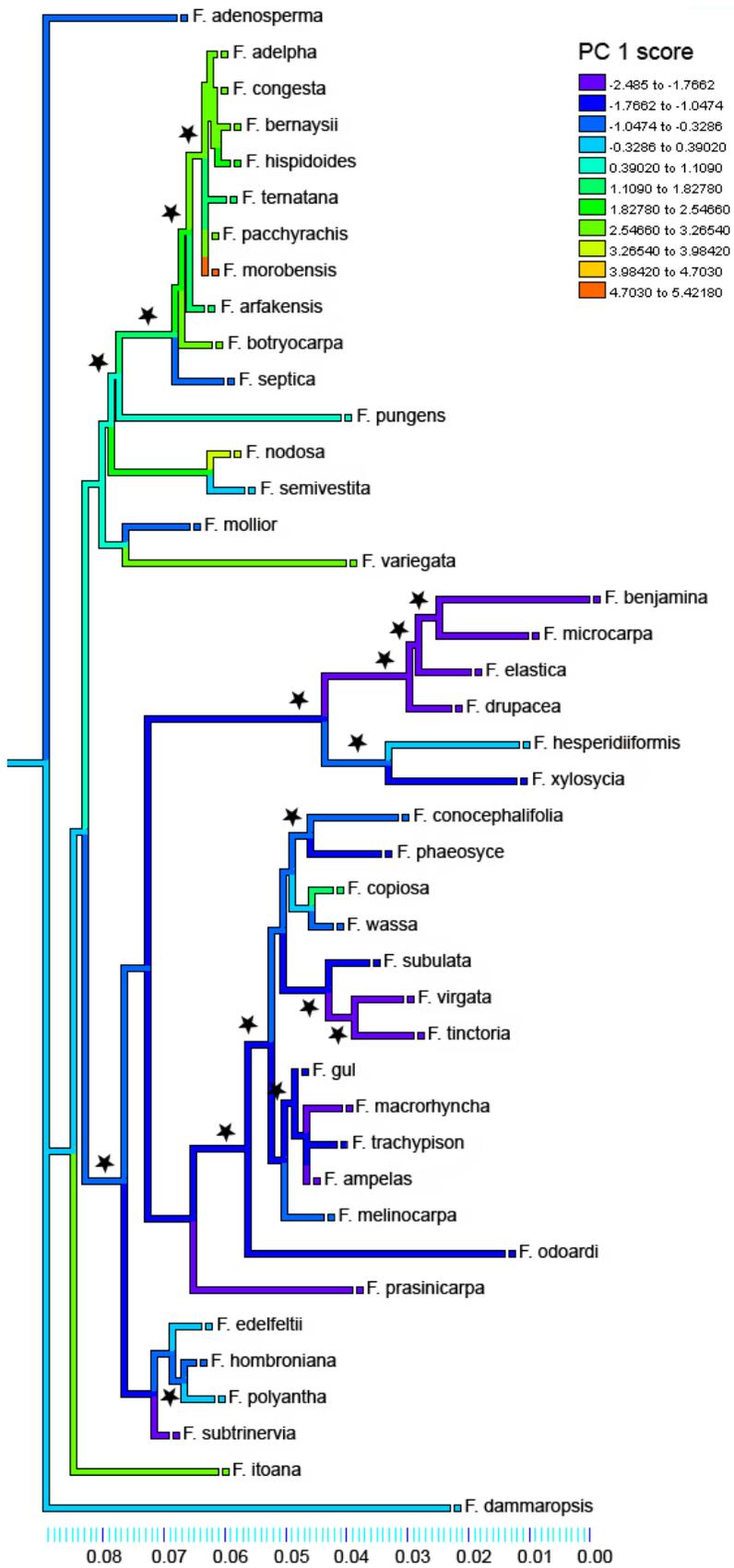
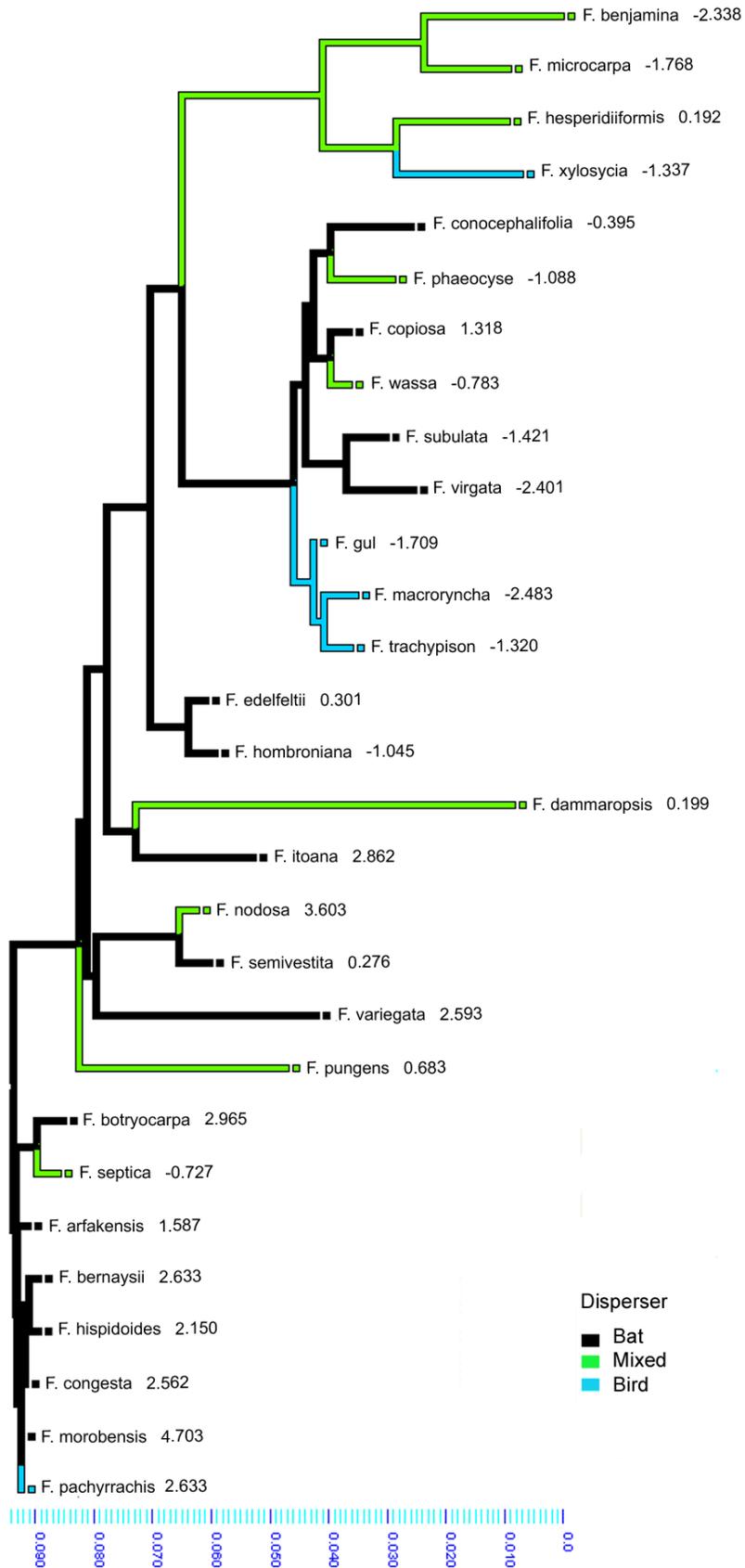
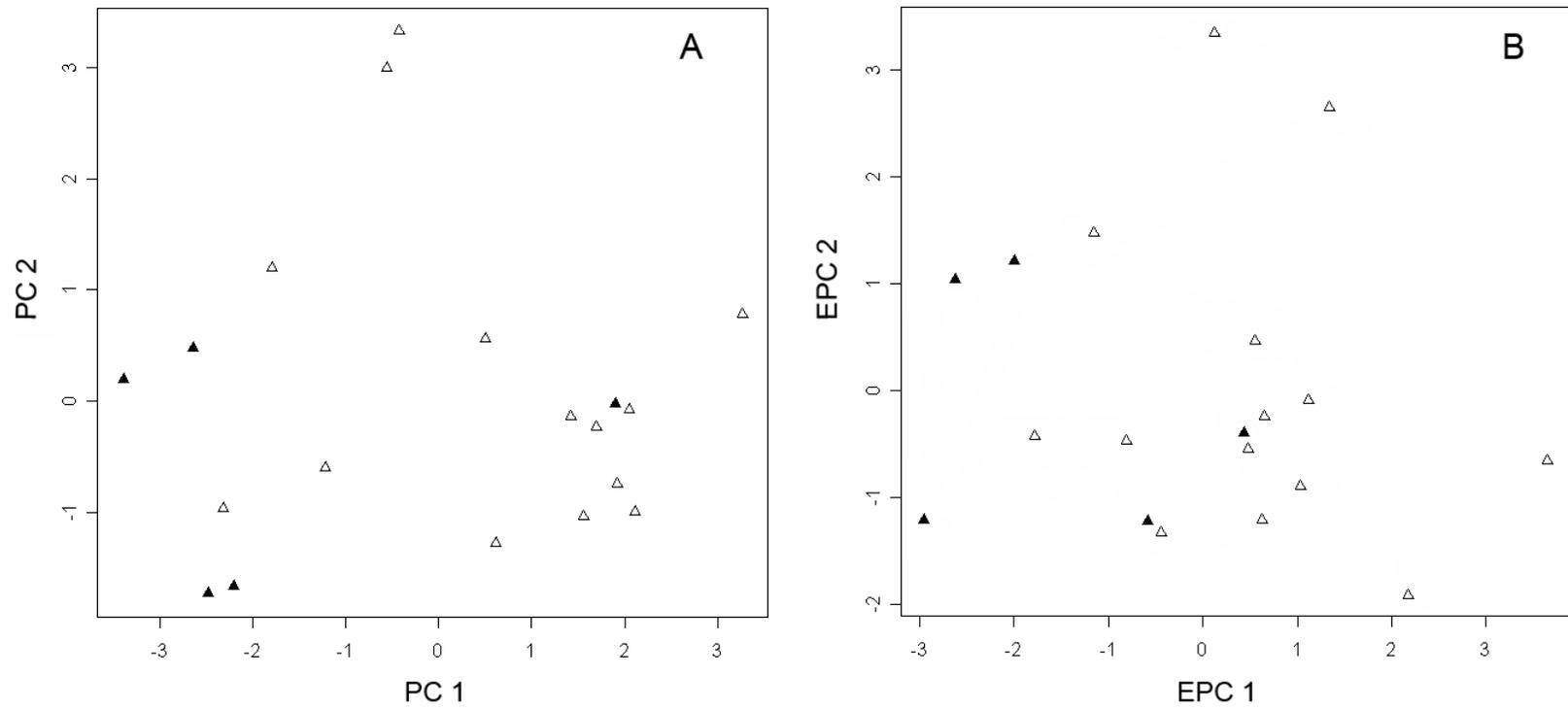


Figure 4-4. Parsimony reconstruction of dispersal mode. The number by the species names is the score on PCA axis 1, which separates figs according to dispersal mode. Disperser mode is shown in key.





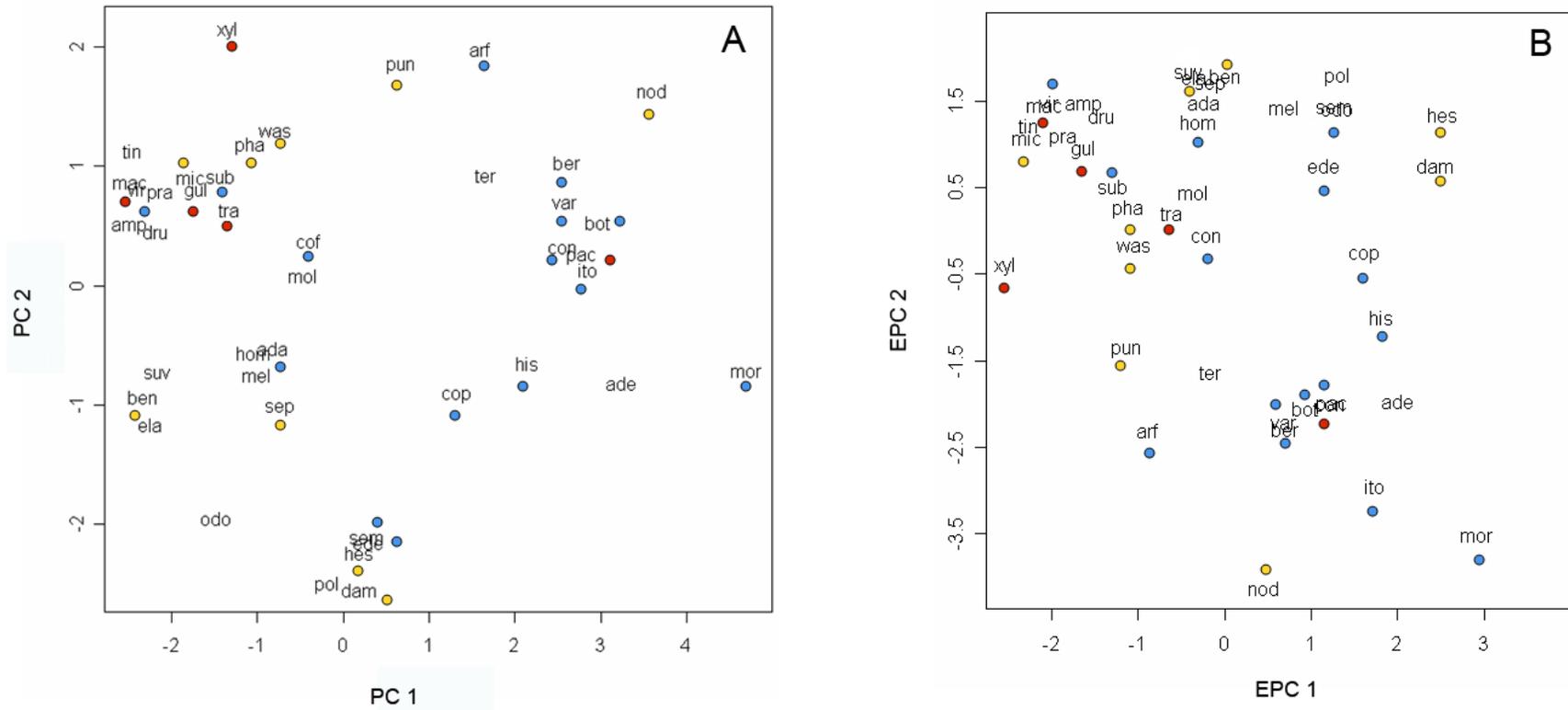


Figure 4-6. Plot of PC 1 vs 2 for the Fruit Trait PCA (A) and EPCA (B) for 42 species of figs. The importance of the original variables in defining the PCs can be seen in Table 4-1. Species names are abbreviated as explained in Table 4-3. Red dots: bird-dispersed species. Blue dots: bat-dispersed species. Yellow dots: mixed dispersal. Fig species without dots had no data on disperser.

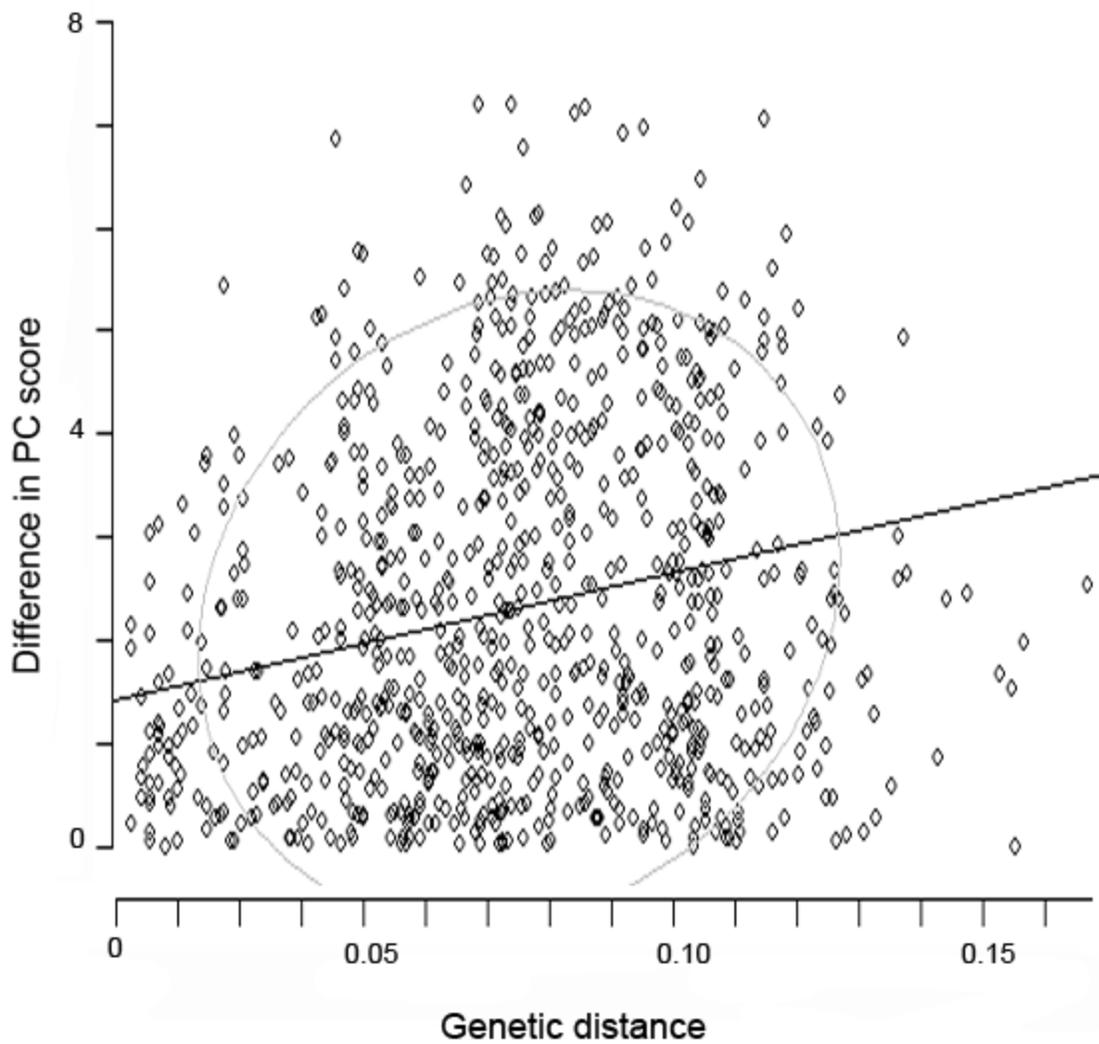


Figure 4-7. Correlation between genetic distance and difference in the score on the PCA axis 1 for all possible pairs among the 42 fig species for which fruit trait data were obtained. The ellipse defines a 95% confidence region.

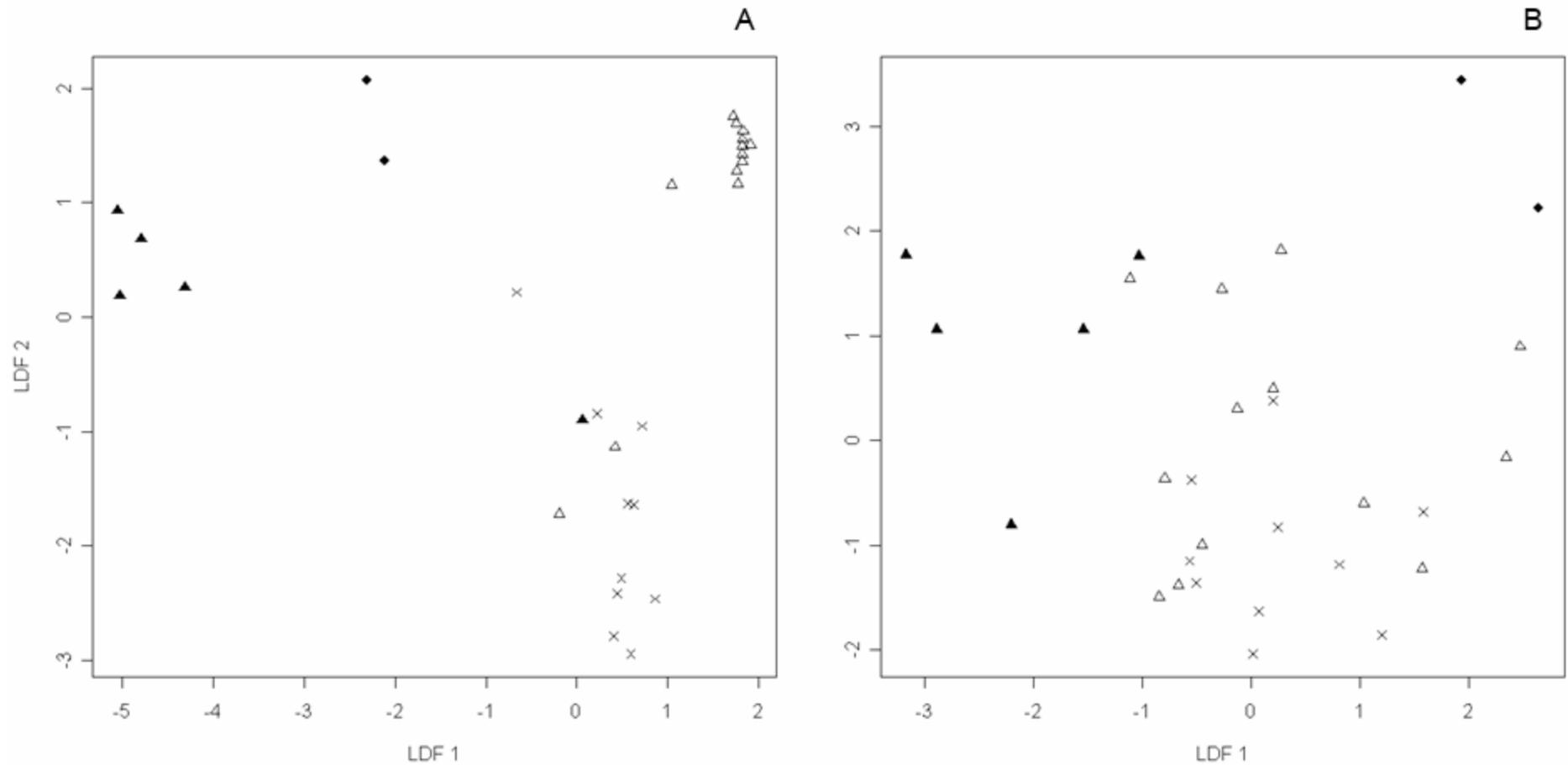


Figure 4-8. Discriminant Functions plot based on (A) original data matrix for 30 *Ficus* species on presence/absence of 73 volatiles compounds, and on (B) the same matrix randomized, keeping the column totals fixed. Symbols represent bird-dispersed (▲), bat-dispersed (△), bandicoot-dispersed (◆), and mixed-dispersed (×).

Table 4-1. Loadings of variables for the Disperser PCA and EPCA. Corresponds to Figure 4-5.

	PC1	PC2	EPC1	EPC2
Diameter	0.336	0.305	0.275	0.208
Softness	-0.383	-0.331	-0.266	-0.204
PC1	0.205	0.236	0.555	0.204
PC2	-0.050	-0.416	-0.171	0.452
PC3	0.153	-0.036	-0.197	0.102
Odor	0.154	0.355	0.423	0.193
Peduncule length	0.346	0.061	0.341	-0.107
Trunk	0.261	-0.529	0.082	-0.574
Branch	-0.366	0.314	0.010	0.363
Contrast against background	-0.437	0.067	-0.316	0.083
Distance from closest leaf	0.368	-0.235	0.273	-0.376
Proportion Variance	0.378	0.172	0.260	0.244

Table 4-2. Loadings of variables for the Fruit Trait PCA and EPCA. Corresponds to Figure 4-6.

	PC1	PC2	EPC1	EPC2
Diameter	0.271	-0.461	0.515	0.013
Softness	-0.308	0.402	-0.445	-0.122
PC1	0.170	-0.559	0.551	0.133
PC2	0.116	0.099	-0.292	0.020
PC3	0.171	0.174	-0.089	-0.012
Odor	0.218	-0.108	0.043	-0.191
Peduncule length	0.370	-0.077	0.301	-0.264
Trunk	0.339	0.312	0.012	-0.476
Branch	-0.402	-0.300	0.090	0.451
Contrast against background	-0.432	-0.116	-0.197	0.294
Distance from closest leaf	0.335	0.234	0.032	-0.587
Proportion of Variance	0.378	0.144	0.214	0.203

Table 4-3. Information on abbreviations used in figures, disperser recorded, and source of information for dispersal mode.

<i>Ficus</i> species	Abbreviation	Disperser	Source
<i>F. adenosperma</i>	ada	no data	
<i>F. adelpha</i>	ade	no data	
<i>F. congesta</i>	con	bat	field work
<i>F. bernaysii</i>	ber	bat	field work
<i>F. hispidioides</i>	his	bat	field work
<i>F. ternatana</i>	ter	no data	
<i>F. pacchyrachis</i>	pac	bird	field work
<i>F. morobensis</i>	mor	bat	field work
<i>F. arfakensis</i>	arf	bat	field work
<i>F. botryocarpa</i>	bot	bat	field work
<i>F. septica</i>	sep	mixed	Shanahan et al 2001
<i>F. pungens</i>	pun	mixed	field work
<i>F. nodosa</i>	nod	mixed	Shanahan et al 2001
<i>F. semivestita</i>	sem	bat	field work
<i>F. mollior</i>	moll	no data	
<i>F. variegata</i>	var	bat	field work
<i>F. benjamina</i>	ben	mixed	Shanahan et al 2001
<i>F. microcarpa</i>	mic	mixed	Shanahan et al 2001
<i>F. elastica</i>	ela	no data	
<i>F. drupacea</i>	dru	no data	
<i>F. hesperidiiformis</i>	hes	mixed	field work
<i>F. xylosyca</i>	xyl	bird	Shanahan et al 2001
<i>F. conocephalifolia</i>	cof	bat	Shanahan et al 2001
<i>F. phaeosyce</i>	pha	mixed	Shanahan et al 2001
<i>F. copiosa</i>	cop	bat	field work
<i>F. wassa</i>	was	mixed	Shanahan et al 2001
<i>F. subulata</i>	sub	bat	field work
<i>F. virgata</i>	vir	bat	field work
<i>F. tinctoria</i>	tin	no data	
<i>F. gul</i>	gul	bird	field work
<i>F. macrorhyncha</i>	mac	bird	field work
<i>F. trachypison</i>	tra	bird	field work
<i>F. ampelas</i>	amp	no data	
<i>F. melinocarpa</i>	mel	no data	
<i>F. odoardi</i>	odo	no data	
<i>F. prasinicarpa</i>	pra	no data	
<i>F. edelfeltii</i>	ede	bat	field work
<i>F. hombroniana</i>	hom	bat	field work
<i>F. polyantha</i>	pol	no data	
<i>F. subtrinervia</i>	suv	no data	
<i>F. itoana</i>	ito	bat	field work
<i>F. dammaropsis</i>	dam	mixed	Shanahan et al 2001

CHAPTER 5
WITHIN SPECIES TEST OF THE DISPERSAL SYNDROME HYPOTHESIS: A STUDY OF
DIOECIOUS FIGS

Introduction

General Background and Goals

Ecological evidence strongly suggests that seed dispersal is important to fruiting plants, to the point that some authors believe the question is no longer *whether* seed dispersal matters, but *when* and *where* it matters most (Howe and Miriti 2000; 2004). If dispersed seeds are indeed more likely to establish than undispersed seeds (Fragoso et al. 2003; Harms et al. 2000), then individual trees that attract more seed dispersers should leave more offspring. Hence, seed dispersers should be a strong selective force on fruit characteristics.

Evidence for the evolutionary role of seed dispersers on fruit traits, on the other hand, is mixed. Many evolutionary ecologists have described patterns in co-occurring traits that they attribute to different selective pressures of seed-dispersing vertebrates (Gautier-Hion et al. 1985; Janson 1983; Lord 2004; Lord et al. 2002; Mack 1993). In particular, they champion the existence of “dispersal syndromes” – non-random combinations of fruit traits that seem to match the foraging capabilities and overall characteristics of seed disperser taxa that forage in disparate ways (McKey 1975; van der Pijl 1969). Other evolutionary ecologists have concluded that dispersal syndromes do not exist or that differences among seed disperser taxa cannot account for them (Fischer and Chapman 1993; Herrera 1987; 1989; 1992; 2002a; Jordano 1995).

The Dispersal Syndrome hypothesis posits that seed dispersers are important for plant reproduction and that fruit traits that attract more seed dispersers are selectively favored. Hence, one should find that dispersal syndromes have evolved independently in unrelated taxa that share similar seed dispersers. Many preceding studies testing this hypothesis have been criticized because they did not control for phylogenetic effects, instead considering each species as an

independent data point (e.g., Gautier-Hion et al. 1985; Janson 1983; Kalko et al. 1996; Korine et al. 2000). Patterns of association between suites of fruit traits and taxa of seed dispersers were interpreted as the result of co-evolution when, in fact, they could be more easily explained by the process of “ecological fitting” –frugivores with preexisting preferences encounter fruits with different traits, but will feed on the fruits that are most easy to find and accessible to them (Felsenstein 1985; Harvey and Pagel 1991; Herrera 1987; 1989; Janzen 1980; 1985; Jordano 1995).

The genus *Ficus* offers an unusual opportunity to test the Dispersal Syndrome hypothesis in a way that tightly controls for phylogenetic effects because some species produce fundamentally different types of “fruits”. (The fruiting structures of *Ficus* are termed syconia, but for simplicity we refer to them as “fruits”.) Functionally female trees produce “seed figs,” which contain viable seeds. These individual trees therefore benefit from attraction of seed dispersers. Functionally male trees produce “gall figs” whose seeds are all consumed by their pollinators’ larvae; they therefore do not benefit from attraction of seed dispersers.

Dumont et al (2004) showed that in one dioecious species, *F. pungens*, fruit bats preferred seed figs over gall figs in feeding trials. Seed figs were larger and had more soluble carbohydrates and fat, and less fiber than gall figs, characteristics that are all energetically important for bats (Dumont et al. 2004). In this study, we expand on Dumont et al’s (2004) work by (1) quantifying frugivore preference for seed figs in natural conditions by videotaping 26 seed figs and 20 gall fig trees of seven species (2) comparing the difference between gall and seed figs of 27 dioecious fig species with respect to five traits that are known to be important in attraction of seed dispersers: fruit size, color, odor, softness, and exposure on the tree (Gautier-Hion et al. 1985; Janson 1983; Kalko et al. 1996; Korine et al. 2000; Lomáscolo et al. 2008; Lord 2004;

Lord et al. 2002; Mack 1993; Schaefer et al. 2006; van der Pijl 1969; Willson and Whelan 1990, Chapter 4, this dissertation); and (3) quantifying at changes in those five traits during the ripening process for gall and seed figs and for figs of monoecious species, to determine whether male trees retain characters from unripe fruits when their fruits are fully mature. We also take a closer look at UV reflectance because it has been shown to attract some seed dispersers (Altshuler 2001). Because most hymenoptera also perceive UV reflectance, and because of the tight mutualism of figs with pollinating wasps, we looked at UV reflectance at the flower, unripe, and ripe stage of seed and gall figs. If UV has a role in frugivore attraction, ripe female figs should show the highest reflectance. If, however, UV is mainly a signal to pollinators, syconia at the flower stage should show the highest reflectance.

Assuming traits that attract seed dispersers are costly to the plant, we predict that only figs with viable seeds (seed figs and figs of monoecious species) should invest in nutrient-rich exocarp because only they would benefit from attracting seed-dispersing frugivores. Specifically, seed figs should be larger, have more pigment and volatile compounds that make them odorous, be softer (higher water to fiber ratio), and have longer peduncles to expose the fruits away from the foliage and trunk. They should also reflect more in the UV range of the spectrum. It has been hypothesized that fruit traits that attract frugivores, especially color and odor, signal ripeness (Sumner and Mollon 2000b) – i.e., that the appearance of fruit changes when seeds are ready to be dispersed. We predict that seed and monoecious figs should show large changes between the unripe and ripe stages, whereas male figs should show relatively smaller changes between unripe and ripe stages.

Natural History of Fig Pollination and Seed Dispersal

Functional dioecy in figs is the result of one of the most tightly coevolved plant-pollinator systems in nature (Janzen 1979; Weiblen 2002), in which typically one species of wasp

pollinates one species of fig. Fertilized female wasps carrying pollen enter the fig inflorescence through a small hole (“ostiole”), often losing their wings and part of their antennae (Wiebes 1979). They then pollinate the female flowers and lay an egg in each flower’s ovary. The wasp larvae develop in the fig ovaries inside the seed coat and consume the endosperm (Weiblen 2002). Once the wasps have completely matured, they exit the seed coat through a hole and mate inside the fig. Females collect pollen from the now mature male flowers and exit the fig through a hole made by the male wasps, which stay and die inside the fig where they emerged. Fertilized female wasps carrying pollen then search for other figs at the correct stage of development to continue their life cycle. In functionally dioecious fig species, female figs produce only female flowers, and they all have long styles that prevent oviposition. Hence, a female fig acts as a wasp trap because female wasps cannot lay eggs in them and cannot exit the fig to search for another. Since female figs produce viable seeds and no wasps, they are called seed figs (Dumont et al. 2004).

Functionally male figs, on the other hand, produce male and female flowers, but all female flowers are short-styled and wasps lay eggs in every one of them, preventing development of any viable seeds; they are called gall figs and produce wasps that disperse their pollen. In monoecious figs, wasps oviposit in some flowers and not others, so that all syconia produce some wasps and some viable seeds.

Fruits of gall and seed figs differ in many traits, including time of development, duration of receptivity of inflorescences to pollinators and duration of fruit development (Patel and McKey 1998), season of ripening (Hill 1967; Patel and McKey 1998), shape and size (Hill 1967; Lambert 1992), color (Dumont et al. 2004; Hill 1967; Lambert 1992), nutrient and fiber content (Dumont et al. 2004, Weiblen, Lomáscolo, and Dumont, unpublished data), and texture (Dumont

et al. 2004; Lambert 1992). These differences are presumably the result of optimizing the male and female functions -- i.e., pollination success in gall figs and frugivore attraction in seed figs (Dumont et al. 2004; Lambert 1992; Patel and McKey 1998).

In this study, we focus on the optimization of the female function of seed figs and explore whether selection pressure by seed dispersers can account for differentiation of gall and seed figs when each is at the ripe stage.

Methods

Study Site and General Specifications

We collected data from September 2004 until December 2005 in a lowland forest in Madang Province, Papua New Guinea, at Ohu Village (145° E, 5° S) (Fig. 4-1). To obtain data on fruit color, color contrast against the background, odor, size, softness, and peduncle length for male and female figs of each species we averaged records from between 3 and 8 individuals per species. For each individual tree, between one and 20 fruits were measured and averaged for all traits.

Quantification of Visits to Figs

We recorded consumption of female and male figs using two video cameras (Sony DCR-HC40), which were set up 3-5m away from the tree and to minimize disturbance, visited only to change tapes or batteries. Videotaping started at 6am and ended at 10:30am, and the again at 6:30pm until 11:00pm, to record both diurnal and nocturnal frugivores. Nocturnal recording was done using an infrared light (Sony HVL-IRH2). We aimed one camera at the ground to record terrestrial frugivores and one either at a branch or at the trunk, depending on where most of the ripe figs were. We recorded 26 female trees (347 hours) and 20 male trees (343 hours) of seven species (*Ficus congesta*, *F. hispidoides*, *F. morobensis*, *F. pachyrrachis*, *F. pungens*, *F. septica*, and *F. virgata*).

Fruit Traits

Fruit color: We quantified fruit color using a USB2000 portable spectrometer (Ocean Optics, Inc) and a PX-2 Pulsed Xenon light source, which took reliable readings between 300 and 740nm. This spectrum includes the range of wavelengths visible by humans, plus the ultraviolet 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 at that angle. The metal stand also blocked external light. We fastened a non-UV filtering microscope slide to the opening of the hole where the sensor was introduced to keep the distance between the end of the sensor and the fruits constant. To obtain an average reflectance spectrum per fruit, we scanned each fruit three times in three different spots on the fruit. We calculated reflectance as the proportion of a certified white reflectance standard (Labsphere). The three spectra for each species were averaged every 5nm. We obtained a quantitative measure of fruit color for each species by doing a Principal Components Analysis (PCA), which summarizes the information for the complete spectrum by finding linear combinations of the original variables (in this case, reflectance intensity every 5 nm) (Clotfelter et al. 2007; Cuervo and Shine 2007; de Ayala et al. 2007; Endler 1990). We did two separate PCAs: one to obtain color variables for the comparison between ripe fruits of male vs. female figs, and a second one to obtain color variables for the comparison between ripe and unripe male, female, and monoecious figs. In both cases, the first principal component (PC) had high positive loadings for all wavelengths throughout the spectrum, and hence it represents fruit brightness, the total amount of light reflected by a fruit (Fig. 5-1). Therefore, light fruits had high scores on PC 1 and dark fruits had low scores. The second PC represents reflectance in the yellow versus the UV-blue wavelengths because it had negative loadings for wavelengths from 300 to around 500nm, and evenly positive loadings from

500 to 740nm. Therefore, yellow fruits had a high positive score on PC 2, whereas fruits reflecting in the UV or blue part of the spectrum had high negative scores. The third principal component represents reflectance in the green versus red and UV-blue range, because it had high negative loadings of wavelengths between 420nm and 610nm. Thus, green fruits have a low score on PC 3 and UV-blue and red (and, therefore, purple) fruits have a high score. These three PCs explained 94% of the variance, and all had eigenvalues greater than one, which means that each explains more variation than any one original variable alone. Although PC 4 and 5 also had eigenvalues higher than one, they only explained 3 and 2% of the variation, respectively. Because reflectance in all parts of the spectrum of interest was summarized by PC 1 through 3, we considered that additional PCs did not contribute sufficiently to the color description of each fig species. Hence, we use only scores on PC 1 through 3 in subsequent analyses.

We calculated color contrasts as the Euclidean distance between the color of a fruit and the color of the structure against which it is exposed to the frugivore. We normalized spectra to the same brightness by dividing the reflectance at each wavelength by the total reflectance for each species. This represents contrasts due to the color of the fruits and not to their brightness, and are called chromatic contrasts (Schmidt et al. 2004a).

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

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 300-740nm.

We analyzed reflectance in the UV range of the spectrum separately because we were interested in including syconia at the flower stage in the comparison. Mean reflectance between 350 and 400nm was calculated and compared between seed and gall figs at the flower, unripe, and ripe stage.

Fruit odor: To collect odor, we collected fruits in the field, brought them to the lab, and placed them inside plastic bags (Reynolds oven baking bags) that had a carbon filter at one end to clean the air coming into the bag, and a filter with Super Q (Alltech) in it to adsorb the volatiles emitted by the fruits. The filter was in turn connected to a vacuum pump (Welch, model no. 2522B-01), which sucked the air through a carbon filter into the bag containing the figs, and through the Super Q filter. All odor collections were run for four continuous hours, and began no more than three hours after the fruits had been collected from the tree. To extract the fruit volatiles from the Super Q filters, we injected methylene chloride through the filters, which eluted the volatile compounds trapped in the Super Q. We used gas chromatography to separate volatile compounds and mass spectrometry to identify some of them. For the purpose of this study, however, only the total amount of volatile compounds is used as a measure of how odorous the fruits were. Because the number of fruits we were able to collect and the size of the fruits varied among trees, we had to standardize the total amount of volatiles by dividing the amount of each volatile contained in the sample by the total surface area of the fruits used to collect odor.

Fruit size, softness, and peduncle length: We measured fruit diameter with calipers to the nearest 0.5 mm and used as a measure of overall fruit size. To measure fruit softness, an arbitrary scale between 1 (hardest, fruit surface did not indent when pressed against fingers) to 4 (softer, fruit surface indented easily when pressure was applied to fruit between the fingers) was determined by the authors and measured by the same person throughout the study. Peduncle length (± 0.5 mm) was measured between the base of the fruit and the point where the peduncle attached to the branch or trunk.

Data Analysis

Frugivore visits to female versus male trees: To test whether female fruits were visited more often than male fruits, we compared the number of visited (12) and not-visited (14) female trees to the number of visited (1) and not-visited (19) male trees using a Chi-square test. Unless a frugivore was specifically seen *not* to eat any fruits the entire time it was in a tree, any frugivore that entered the tree was assumed to have been attracted to the tree and its presence was recorded as a visit. A tree was recorded as visited if at least one frugivore entered the tree.

Difference in fruit traits of seed and gall figs: To identify the variables that distinguish gall and seed figs, we performed a Discriminant Function analysis (DFA) on seed and gall figs of 25 species. Discriminant Function analysis finds the combination of traits that best discriminates between the groups of interest, in this case, seed and gall figs (i.e., two groups).

Changes from unripe to ripe stage in monoecious and dioecious figs: To identify how traits changed from the unripe to the ripe stages, and to determine whether these changes are similar between figs with viable seeds (female and monoecious figs) and those without viable seeds (male figs), we ran another DFA on the data including unripe monoecious, unripe seed, unripe gall, ripe monoecious, ripe seed, and ripe gall figs (i.e., six groups). For both DFAs, all variables were standardized to have a mean of zero and standard deviation of one because fruit variables were measured in different scales and had differences of up to three orders of magnitude. We tested the significance of the discrimination by calculating Wilk's Lambda, which represents the proportion of the variance not explained by the predictor variables. This test, however, does not inform which groups differ from which. To analyze which groups in particular could be considered different, we performed an ANOVA on the Linear Discriminant Function (LDF) that summarized most of the variation among the groups and performed a Tukey test. We were particularly interested in comparing: 1. male fruits (not consumed by frugivores)

and female figs (consumed by frugivores); 2. female and monoecious fruits, which are both reportedly consumed by frugivores; 3. ripe male and unripe male fruits, to see whether males retain the characteristics of unripe fruits; 4. unripe male, female and monoecious figs, to see whether the differentiation between fruits consumed by frugivores and those not consumed by frugivores starts at ripening or whether these fruits are different from earlier on.

Another way we tested the predictive value of the linear discriminant functions is the predictive classification of cases, which is done using Baye's Rule to calculate the posterior probabilities of each case of being in each group, based on each case's discriminant score. The higher the percentage of species that were well classified (e.g., known females correctly classified as a female) the higher the predictive value of the LDF.

UV reflectance: We used Mann-Whitney U tests for pair-wise comparisons of mean UV reflectance by figs of different sex and maturity stage. A sequential Bonferroni correction (Rice 1989) was used to calculate the actual critical p-value in multiple comparisons.

Results

Frugivore Visits to Female Versus Male Trees

Of the 26 female trees videotaped, 12 were visited by frugivores. Of the 20 male trees videotaped, one was visited by a frugivore. Visits to female trees were significantly higher than visits to male trees ($\chi^2 = 7.52$, d.f. = 1, $p = 0.006$).

Difference in Fruit Traits of Seed and Gall Figs

One linear discriminant function (LDF) was sufficient to separate female from male figs as two groups can only be separated in one dimension (Fig. 5-2). In order of importance, as measured by the loadings of variables on the LDF (Table 5-1), female fruits were softer, had more volatile compounds, were larger, darker, and contrary to predictions, reflected more in the green part of the spectrum than did male figs. Female figs were also more contrasting against the

foliage and had shorter peduncles, but the loadings of these variables were quite low and, hence, less important in discriminating between male and female figs. Discrimination of female and male figs based on the fruit variables mentioned was statistically significant (Wilk's $\lambda = 0.339$, numerator d.f. = 8, denominator d.f. = 41, $p < 0.0001$). When the scores of female and male trees of the same species are compared, all except one species (*F. dammaropsis*) show a higher score for females than for males (Fig. 5-3). Cross classification resulted in 94% of the cases correctly classified.

The difference in fruit traits for gall and seed figs is present in two different clades that seem to have evolved dioecy independently (Fig. 5-4)

Changes From Unripe to Ripe Stage in Monoecious and Dioecious Figs

The fruit variables included in this study can significantly discriminate between figs of different sex and ripening stage (Wilk's $\lambda = 0.043$, numerator d.f. = 40, denominator d.f. = 473.56, $p < 0.0001$) (Fig. 5-5). In general, monoecious and female ripe fruits scored highest on LDF 1, followed by male ripe fruits, and then by all unripe fruits. According to the variable loadings on LDF 1 (Table 5-2), this means that monoecious and female fruits are softer, darker, and had higher reflectance in the red and UV-blue part of the spectrum than did male ripe fruits and all unripe fruits. Monoecious and female ripe fruits were also slightly more odorous and had longer peduncles, but these variables had low loadings and are therefore less important in discriminating between the groups than are softness, brightness and green reflectance. The rest of the variables had even lower loadings on LDF 1. A post-hoc Tukey test showed that unripe figs from different sexes were not significantly different from each other, but were significantly different from all ripe figs. Ripe gall figs differed significantly from all unripe figs and from ripe seed and monoecious figs. Monoecious and seed figs did not differ from each other but did differ from all other figs (Fig. 5-6).

UV Reflectance

The number of figs included in each comparison and mean reflectance for each sex and maturity stage are summarized in Table 5-3. Against our prediction under the hypothesis that UV signals frugivores, mean UV reflectance by ripe gall figs was significantly higher than UV reflectance by ripe seed figs ($W = 951, p = 0.00007$). Reflectance of UV light does not seem to have an exclusive role in signaling to wasps either, as reflectance did not differ between the flower and the ripe stage for both sexes pooled together ($W = 482, p = 0.87$). No difference was detected between gall and seed syconia at the flower stage, although this may be due to the small sample size. UV reflectance was higher for unripe seed figs than for ripe seed figs ($W = 1321, p = 0.003$), but this difference was not present between unripe gall figs ($n = 26, \bar{x} = 2.35$) and ripe gall figs ($W = 292, p = 0.22$).

Discussion

Seed dispersers were attracted to seed figs, which showed many of the characteristics that were predicted to attract frugivores. As predicted, seed figs are softer than gall figs, which probably facilitates frugivore consumption (Dumont 1999; Dumont and O'Neal 2004); more odorous, which is especially important for mammalian frugivores (Hodgkison et al. 2007; Korine and Kalko 2005); larger; and darker. Contrary to expectation, seed figs reflected more in the green and less in the red and UV-blue range of the spectrum than did gall figs. This is puzzling, at least at first, because only seed figs showed intense red, purple, or black coloration. A potential explanation is that many red seed figs showed a dark red coloration, whereas the gall figs for the same species showed a lighter red, almost pink, or an orange-red (e.g., *F. itoana*, *F. conocephalifolia*, *F. dammaropsis*, *F. macrorrhyncha*, *F. odoardi*, *F. phaeosyce*, and *F. wassa*). This results in the overall reflectance in the red spectrum being higher in the lighter gall figs than in the darker seed figs. In other words, the main difference between these gall and seed figs is

not so much which part of the spectrum reflects most light (which is what the PC variables represent), but instead the saturation of the red coloration. Saturation, also called chroma, represents the “purity” of a color, and is measured by the steepness of the reflectance spectrum, in this case, in the red part of the spectrum (Endler 1990). Saturation of a pink or orange fruit is lower than that of a red fruit, but overall reflectance in the red part of the spectrum can still be higher if the pink or orange fruit is brighter. Unfortunately, we did not quantify saturation.

An alternative explanation, non-exclusive of the previous one, is that the high positive loading of PC 3 (“UV-blue-redness” in Table 1) on LDF 1 is more representative of reflectance in the UV range than in the red range. When reflectance in the UV range is investigated in more detail for all species in this study, gall figs reflect more in this part of the spectrum than female figs. Moreover, unripe seed figs reflect more in the UV than ripe seed figs. These two results in conjunction may result because some fruits have a waxy layer at the unripe stage that protects them from desiccation, which often wears off as time passes and the fruits ripen (H. M. Schaefer, pers. comm.). Because gall figs often detach from the tree as soon as the wasps emerge whereas seed figs stay attached until a frugivore eats them or they start rotting, the gall figs we collected may have been “younger” than the seed figs we collected and hence have retained more of the waxy layer than did the seed figs. UV reflectance may also be selected for at the receptive stage to attract pollinating wasps and be retained as the fig matures. This is supported by our results showing that UV reflectance for gall and seed figs was similar at the flower stage, as both are under strong selective pressure to attract pollinating wasps. However, this would not explain the higher UV reflectance in gall figs compared to seed figs (Dumont et al. 2004). What is most puzzling, however, is the lack of difference in UV reflectance between figs at the flower and ripe stage. The trend in UV reflectance as the fig goes from the flower, to the unripe, and to the ripe

stage seems to differ between seed and gall figs (Table 5-3). The number of figs at the flower stage is very low compared to the number of ripe figs and therefore the results for this comparison are best considered inconclusive.

Although contrast against the background showed the predicted trend, i.e., seed figs contrasting more against the foliage than gall figs, it did not appear as important as the previously discussed variables in discriminating between the groups. This may be because, even though some of the seed figs were red and purple (which have the highest contrast against the foliage, Schmidt et al. 2004a), more than a third of the seed figs were green when ripe and had a very low chromatic contrast against the foliage. Although peduncles varied highly among fig species (Chapter 5, this dissertation), it was less important in differentiating between seed and gall figs.

Figs with viable seeds were predicted to be under selective pressure by seed dispersers and were indeed significantly different from all types of figs without viable seeds (i.e., gall and unripe figs of both sexes). Figs with viable seeds were softer, darker, and reflected more in the red and UV-blue part of the spectrum than all other figs, as represented from their higher scores on LDF 1. To a lesser degree, they were also more odorous and larger, and had longer peduncles than all other figs. Contrary to my predictions, gall figs were significantly discriminated from unripe figs, which means that they do not necessarily retain all the characteristics of unripe figs. Even though they do not have viable seeds and do not need to signal to seed dispersers, their traits do change during the ripening process, although to a lesser extent than do figs with viable seeds, as seen from their intermediate mean score on LDF 1 (Fig. 5-5).

A limitation of this study, and possibly the reason why some of the predictions did not hold, is that we established predictions for the differences between seed and gall figs based on

the potential selective pressure of seed dispersers in general even though, for example, birds and mammals may exert very different, and potentially even conflicting selective pressures on different fig species (Chapter 4, this dissertation). It is especially revealing that the seed figs that were exclusively consumed by birds (*F. gul*, *F. macrorrhyncha*, *F. pachyrrachys*, and *F. xylocisia*) (Chapter 4, this dissertation) have a higher average score on LDF 1 than the mean for all seed figs (5.21 vs. 3.61, respectively), whereas the mammal dispersed seed figs (*F. adenosperma*, *F. arfakensis*, *F. bernaysii*, and *F. botryocarpa*, *F. conocephalifolia*, *F. congesta*, *F. copiosa*, and *F. hispidioides*, *F. itoana*, *F. morobensis*, *F. variegata*, and *F. virgata*) (Chapter 4, this dissertation) average lower than the mean for all seed figs (3.47 vs. 3.61, respectively). This apparent separation within the group of seed figs suggests that bird- and mammal-dispersed figs have different characteristics. The within-group differences are insignificant compared to the between group differences, as shown by the highly significant results for the DFAs in this study. Comparing gall and seed figs while distinguishing between bird- and mammal-consumed figs should be particularly enlightening. Birds are diurnal frugivores and have acute color vision (Bowmaker et al. 1997; Bowmaker and Hunt 2006; Hart 2001) that they use to find fruit. On the other hand, all frugivorous mammals in Papua New Guinea are either nocturnal or crepuscular/nocturnal, and must therefore rely more heavily on senses other than vision to find fruit. Odor, for example, has been reported as important for nocturnal mammals of other regions when searching for fruit (Hodgkison et al. 2007; Korine and Kalko 2005; Thies et al. 1998).

A particularly interesting comparison to elucidate the difference in gall and seed figs for mammal- and bird-dispersed figs would be to examine the difference in traits between gall and seed figs for bird- and mammal-dispersed figs. Trait values can be subtracted between gall and seed figs of each species, and the differences used as variables on a DFA for bird-dispersed

versus mammal-dispersed figs. we predict that in bat-dispersed figs, the major differences between gall and seed figs will be the amount of volatile compounds produced, given that bats are mostly crepuscular/nocturnal and cannot rely so much on visual cues, and in structures such as the peduncle that expose the fruits away from the foliage and trunk, given that most of the smaller flying foxes common in Papua New Guinea either grab the fruit in flight or land for a few seconds on the fruit itself and then fly away with the fruit in their mouth (pers. obs). In bird-dispersed figs, on the other hand, major differences in gall and seed figs should be in the production of pigments that make the fruits visually conspicuous by contrasting against the foliage, given that birds have such acute color vision (Bowmaker et al. 1997; Bowmaker and Hunt 2006; Hart 2001; Schaefer et al. 2006; Schaefer et al. 2007; Schmidt et al. 2004b), and in fruit softness, given that birds do not have teeth and can only use their bills to handle the fruits.

Although we do not know the proximate mechanism that determines the differences between seed and gall figs, our results suggest that the ultimate mechanism is selective pressure by seed dispersers. Seed figs show combinations of fruit traits that were predicted (Chapter 4, this dissertation) and shown (this Chapter) to attract seed dispersers, while these attractive trait combinations are not present in individuals that do not benefit from attracting seed dispersers. This is true for both groups of figs that have independently evolved dioecy (Figure 5-4), rendering further support to the adaptive significance of the difference between seed and gall figs. Despite not distinguishing between frugivore guilds, the differences found among gall figs and figs with viable seeds (monoecious and seed figs) support the hypothesis that, in general, frugivores are an important selective force in shaping the evolution of fruit traits in figs.

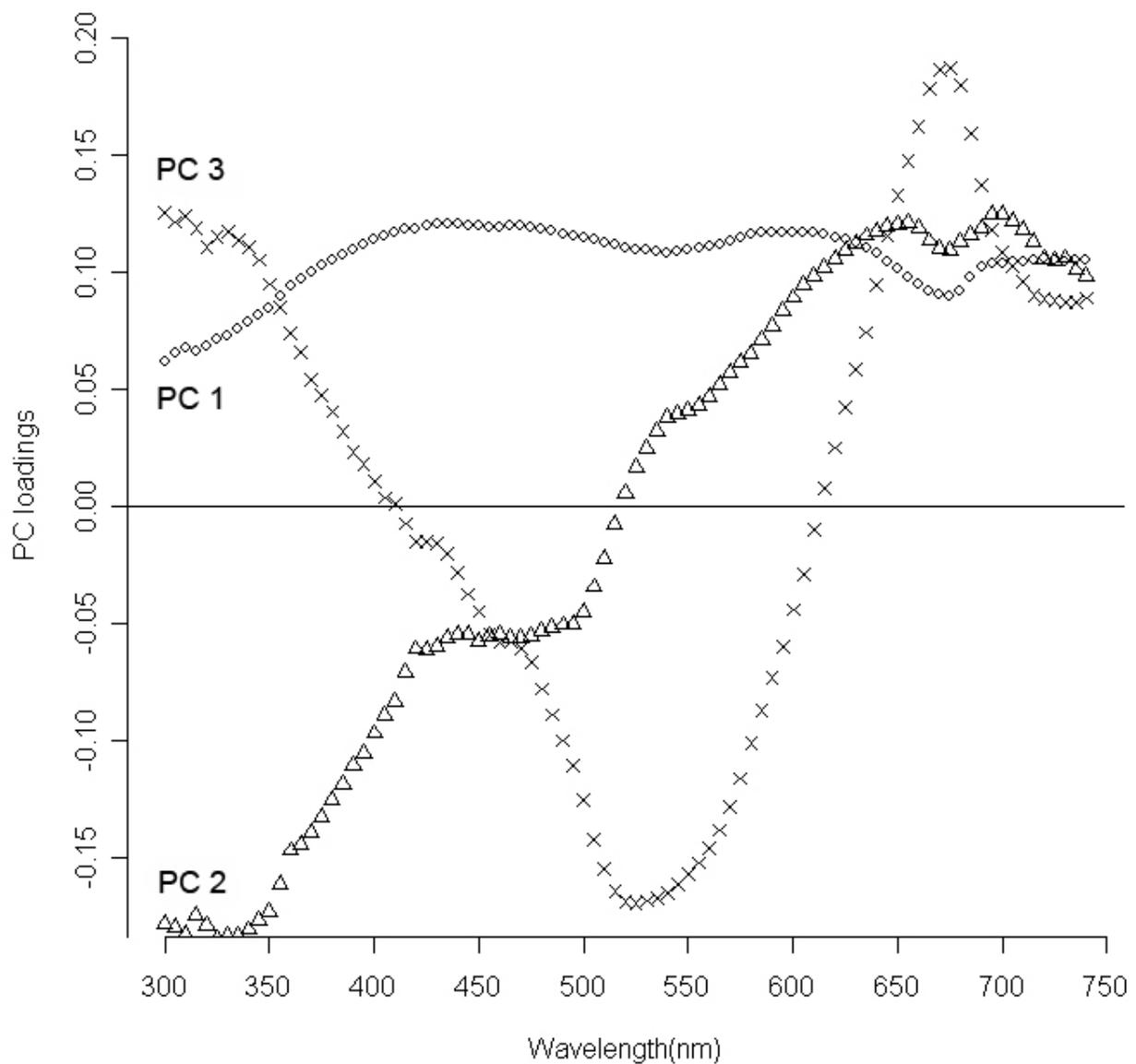


Figure 5-1. Loadings of the first three principal components (PC) on fruit reflectance spectra from 300 to 750nm, measured at 5nm intervals. PC 1 represents variation in mean brightness, PC 2 in green versus red coloration, and PC 3 in UV and blue coloration.

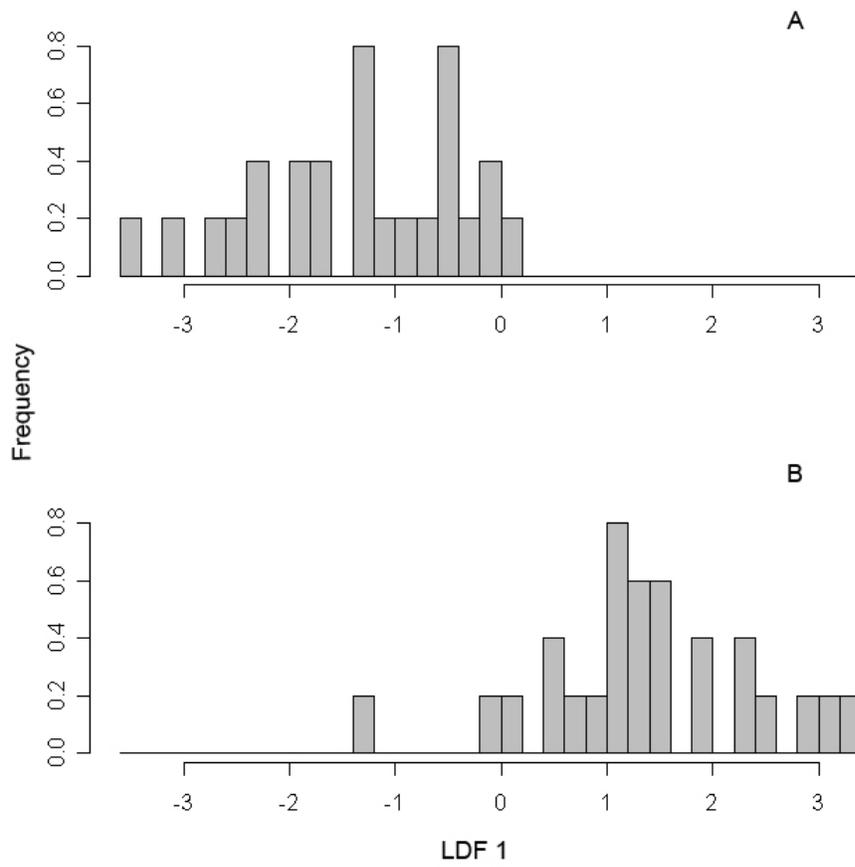


Figure 5-2. Plot of Discriminant Function analysis. (A) Female figs (B) Male figs

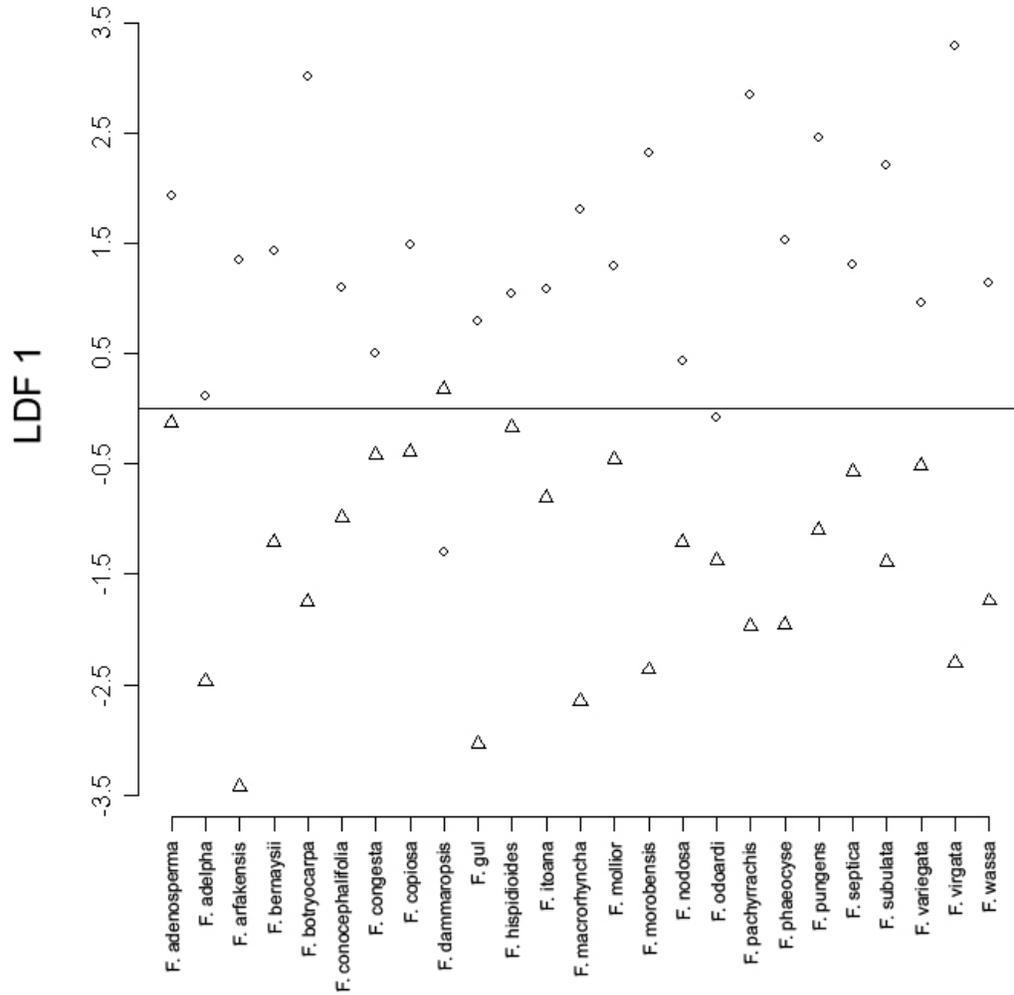
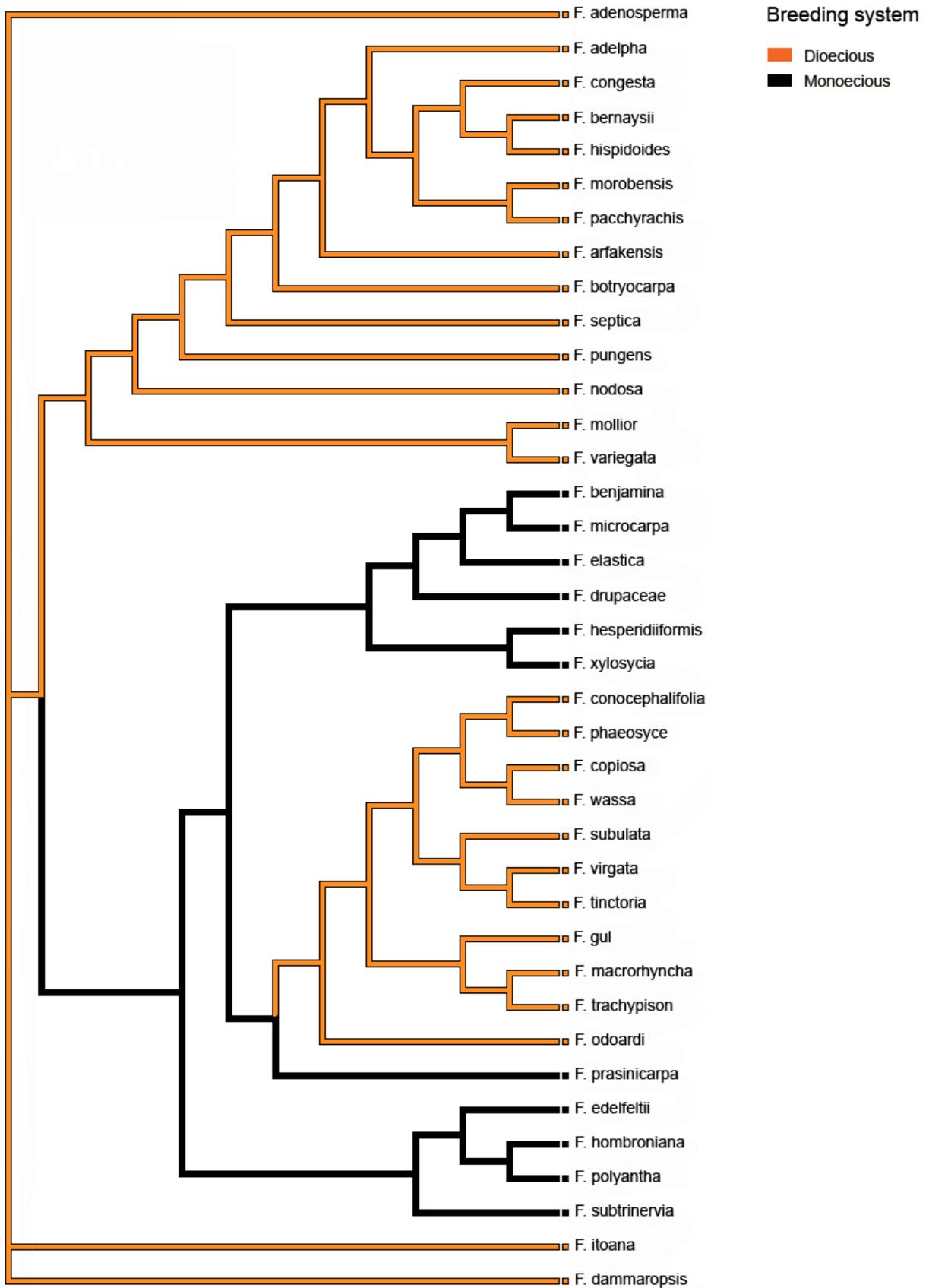


Figure 5-3. Scores on the linear discriminant function (LDF 1) resulting from the discriminant analysis of female and male figs. For each species, the paired scores on the LDF 1 for female (○) and male (△) individuals are plotted. Note that for all species, except one (*Ficus dammaropsis*) female trees score higher than male trees. The horizontal lines points to a value of zero on the LDF score, and is drawn simply as a point of reference.

Figure 5-4. Maximum Likelihood phylogeny of the species included in the seed-gall fig comparison. From the mapping of the breeding system, it is apparent that dioecy evolved twice in this group of *Ficus*. This phylogeny was obtained as explained in Chapter 4, and species that were not included in the analyses done in this chapter were trimmed off the tree.



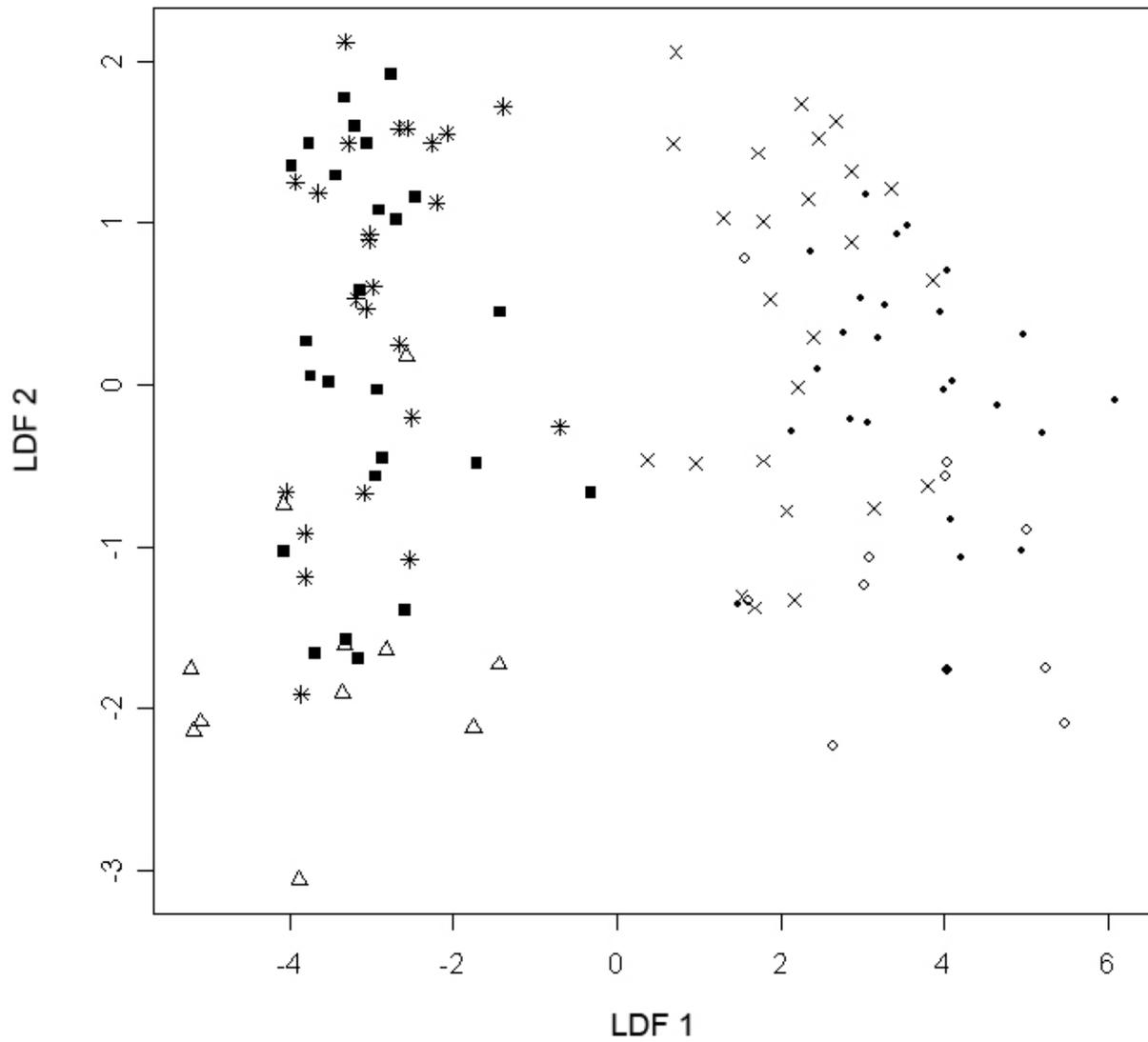


Figure 5-5. Discrimination among figs of different sex and maturity stages using a Discriminant Function analysis. Symbols represent: monoecious ripe (\circ), female ripe (\bullet), male ripe (\times), monoecious unripe (Δ), female unripe (\blacksquare), and male unripe ($*$) figs.

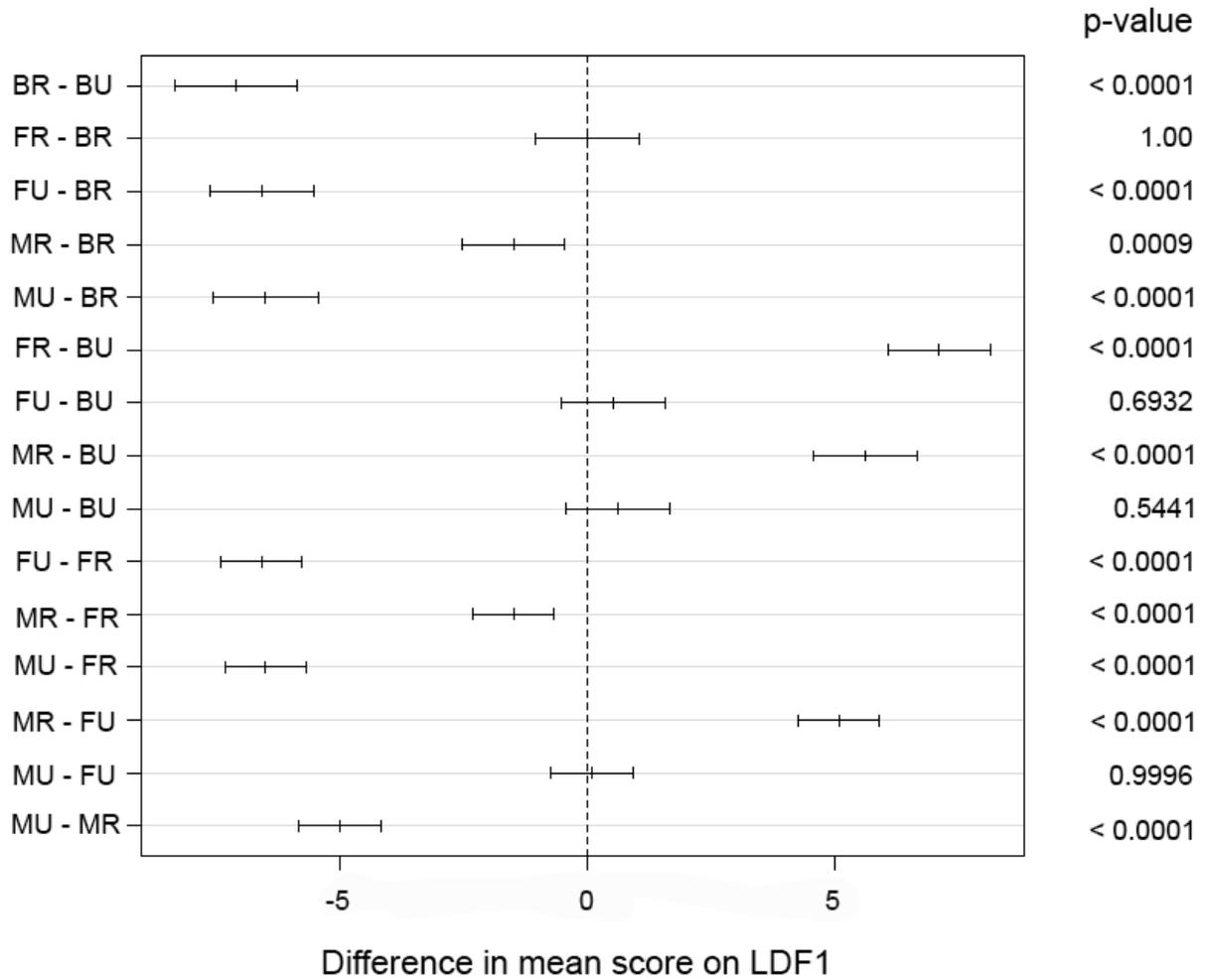


Figure 5-6. Pair-wise comparisons among fig groups of different sex and maturity stages using Tukey tests. BR: monoecious ripe, BU: monoecious unripe, FR: female ripe, FU: female unripe, MR: male ripe, MU: male unripe. The only groups whose difference is not significantly different from zero were female and monoecious ripe figs, and all pairwise comparisons of unripe figs.

Table 5-1. Discriminant Function Analysis of female versus male figs using eight fruit variables. Variables with higher loadings (above 0.3) are marked with an asterisk

Variable	Loading on LD 1
softness	*-1.239
diameter	*-0.551
Odor	*-1.113
Contrast	-0.171
PC 1 (brightness)	*0.763
PC 2 ("yellowness")	*0.338
PC 3 ("UV-blue-redness")	*0.366
Peduncle length	0.182

Table 5-2. Discriminant Function Analysis of female, monoecious and male figs at different maturity stages using eight fruit variables. Variables with higher loadings (above 0.3) are marked with an asterisk

Variable	Loading on LD 1	Loading on LD 2
softness	*3.026	0.102
diameter	0.199	0.083
Odor	0.202	-0.248
Contrast	-0.120	*-1.017
PC 1 (brightness)	*-0.321	-0.027
PC 2 ("yellowness")	-0.145	*-0.337
PC 3 ("UV-blue-redness")	*0.360	0.056
Peduncle length	0.261	-0.044
Proportion of variation	0.92	0.05

Table 5-3. Mean UV reflectance for fig species of different sex and maturity stages

Sex	Maturity stage	Observations	Mean UV reflectance
Female	flower	7	1.09
Female	unripe	44	1.66
Female	ripe	44	1.06
Male	flower	6	2.39
Male	unripe	26	2.35
Male	ripe	28	2.04

CHAPTER 6

GENERAL CONCLUSIONS

This study contributes to the advancement of the field of plant-frugivore interactions because we help resolve a long standing paradox: although animals facilitate plant reproduction through seed dispersal, they do not seem to shape plant evolution. We show that a large portion of the variation in fruit characteristics is unexplained by phylogeny, and that the correlated evolution of seven traits point to seed dispersers as the main selective force explaining this variation. Our study's success in detecting the evolutionary influence of frugivores on fruit traits likely results from our ability to avoid these limitations of earlier work by: 1. focusing at the species level, where most evolutionary meaningful variation occurs; 2. tightly controlling for phylogenetic effects with a phylogeny, the closest approximation to the true evolutionary history of the taxa under study, and by doing a within-species study; 3. studying groups of species on both local and geographic scales; 4. using quantitative measures of fruit color and odor, important signals to frugivores that had previously been ignored or mismeasured, and taking into account the frugivore's perspective on color; 5. incorporating a "holistic" view of Dispersal Syndromes by analyzing all fruit variables together, which we believe better reflects how dispersers search for and assess fruit; and 6. defining Dispersal Syndromes more narrowly and grouping dispersers according to their similar potential to select for fruit characteristics.

One method to test for natural selection in the field is to search for correlated evolution among traits that, in combination, offer higher fitness to the individual bearing them than any other combination. Convergence of multiple traits of distantly related species form strong evidence that the traits do not have a neutral adaptive effect (Endler 1986). We have shown that dispersal syndromes are suites of traits that have converged in distantly related species.

Moreover, we show that these suites of traits can be predicted based on the behavioral and anatomical characteristics of the animals that feed on the fruits. We generate testable predictions of the potential frugivores of different fig species that can be used to test our results. More importantly, these predictions can be generalized to other plant taxa, and to other plant-animal interactions, such as pollination, for which fruit and floral traits can be quantified.

Although arguably to a lesser extent, students of pollinator-plant interactions also ponder the question of how specialized this interaction really is, and whether pollinators affect the evolution of flowering plants (Bluthgen et al. 2007; Johnson and Steiner 2000; Vázquez and Aizen 2004; Waser et al. 1996). Fruit and flower traits have been hypothesized to act as signals in plant-animal communication (Chittka and Raine 2006; Goff and Klee 2006; Schaefer et al. 2008; Schaefer and Schaefer 2007; Schaefer et al. 2004; Sumner and Mollon 2000b) and therefore their importance must be assessed from the perspective of the animal to which the signal is targeted. That an animal differentially perceives signals directed to it versus directed to other animals is an important assumption of plant-animal interactions studies. Pollinators, for example, seem to respond to color cues to locate flowers (Bradshaw and Schemske 2003; Chittka and Raine 2006; Gumbert et al. 1999). In our test of this assumption in fruit to frugivore signaling, we show that the color of bird and primate-dispersed fruits can be perceived as different from the bird's and primate's perspective. This result confirms that fruit color can be used as a signal by plants that may differentially benefit from bird or primate consumption of their fruits. A similar assessment of other fruit traits, such as odor signals, would be needed, but might be immensely more challenging, as assessing perception of odor by animals would likely require many more "dimensions" than for color (Mamlouk and Martinetz 2004), and these dimensions are far from being completely identified (Chittka and Brockmann 2005).

With the exception of a few thoroughly studied cases of tight coevolution between plants and their mutualistic pollinators (Janzen 1979; Smith et al. 2008; Thompson and Pellmyr 1992) or seed dispersers (Amico and Aizen 2000; Reid 1991) mutualistic plant-animal interactions seem to be quite generalized (but see Fenster et al. 2004; Fuentes 1995; Herrera 1985; 1998; Johnson and Steiner 2000; Waser et al. 1996). Moreover, it has been argued that the distribution in plant-animal mutualistic networks can be reasonably well explained by stochastic processes (Vázquez 2005). However, the general observation that pollinator-plant systems are more specialized relative to frugivore-plant systems seems to hold (Bluthgen et al. 2007). The potential for tight coevolution between one species of seed disperser and one species of plant is low because, among other things, most plants benefit from the dispersal services of many different frugivores and, any one frugivore consumes a wide range of fruits. Also, generation times and historical species turnover rates are very different between frugivores and plants (Herrera 1985). Moreover, populations of the same species interact with different species along their geographic range (Gomulkiewicz et al. 2000; Rey et al. 2006; Thompson 1994; 1999). For all these reasons, it has been argued that frugivores and plants undergo, at best, diffuse coevolution — which occurs when groups of species interact with each other (Janzen 1980).

We believe diffuse coevolution should not be viewed as a hopelessly messy evolutionary relationship. If one can identify groups of species that interact in a consistent and distinctive way (e.g., a guild of seed dispersers that exerts similar selective pressure on the plant), and if one objectively defines what traits these interactants are most likely to exert selection upon, then one can make specific predictions on the potential for coevolution. We have focused only on one side of the interaction between frugivores and plants -- the role of seed dispersers in the evolution of fruit traits. The other side -- the role of plants in the evolution of frugivore anatomy

and physiology of visual, and olfactory systems, or on its foraging behavior -- remains to be tested (Dominy et al. 2003; Osorio and Vorobyev 1996; Peichl 2005; SurrIDGE et al. 2003).

It can be argued that modern day frugivores may not represent the fauna that historically interacted and exerted selection on figs. Figs have almost certainly 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, figs as we know them today are likely to have interacted with species of bats and birds that are similar to those seen today. Both the origin of modern-day *Ficus* and Megachiroptera seem to trace back to the same historic epoch and region. The origin and diversification of the fig family, Moraceae, is most parsimoniously assumed to be in Eurasia, from where it would have dispersed to the Southern hemisphere through land connections (Zerega et al. 2005). Modern day figs diversified some time during the Eocene (Zerega et al. 2005) and this is likely to have occurred in the Asian-Australasian region as this is the center of distribution for many fig sections, and where the highest fig diversity is found, worldwide (Berg 1989). Megachiroptera are also thought to have originated in the South East Asian-Melanesian region (Giannini and Simmons 2003). Fully flighted bats are also known from the Eocene and, although no fossils remains of Megachiroptera have been found in the region, recent ancestor of their closely related clade, the Microchiroptera, are known from the Australia-New Guinea region (Bonaccorso 1998). Also, the oldest Megachiroptera fossil found was in Thailand (Ducrocq et al. 1993). The fig species found in New Guinea likely interacted with modern day birds since their origin in Eurasia, as birds evolved much earlier than figs, at least 150 mya and were widespread. However, they likely only came into contact with the birds with which they interact today only in the Pleistocene through a land bridge that connected the island with Australia during the last Ice Age. The island of New Guinea is comprised of a section of the

northern part of the Australian continent to the South, and a series of islands that collided with the Australian continent in the past 40 m.y. to the North (Audley-Charles 1991). Although most of the flora of New Guinea have closest affinities with Southeast Asia, most New Guinean birds, are of Australian origin (Beehler et al. 1986). Because the characteristics on which we based the potential for selection by birds on figs were already present in earlier birds, we consider it unlikely that selection by birds changed significantly in the evolutionary history of figs.

Returning to the first point made in the Introduction, just as it has been argued that incorporating positive interactions can drastically change core concepts in ecological theory (Bruno et al. 2003), we show that, at least one positive interaction – that between frugivores and fruiting plants – can help explain an important evolutionary process – the diversification of fleshy fruits. We argue that studies on positive interactions can help us understand processes that are important for the origin and maintenance of biodiversity.

In our humble quest to identify the role of phylogeny versus frugivores in shaping the evolution of fruit characteristics in figs, we hope to have contributed, even if a little, to the understanding of the evolutionary relationships in the “entangled bank, covered with plants of many kinds” interacting with different animals “dependent on each other in so complex a manner” (Darwin 1859).

LIST OF REFERENCES

- Adams SB, Hamel PB, Connor K, Burke B, Gardiner ES, Wise D (2007) Potential roles of fish, birds, and water in swamp privet (*Forestiera acuminata*) seed dispersal. *Southeastern Naturalist* 6:669-682
- Agosta SJ (2006) On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. *Oikos* 114:556-565
- Alcantara JM, Rey PJ (2003) Conflicting selection pressures on seed size: evolutionary ecology of fruit size in a bird-dispersed tree, *Olea europaea*. *Journal of Evolutionary Biology* 16:1168-1176
- Alfaro ME, Zoller S, Lutzoni F (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* 20:255-266
- Altshuler DL (2001) Ultraviolet reflectance in fruits, ambient light composition and fruit removal in a tropical forest. *Evolutionary Ecology Research* 3:767-778
- Altshuler DL (2003) Flower color, hummingbird pollination, and habitat irradiance in four neotropical forests. *Biotropica* 35:344-355
- Amico G, Aizen MA (2000) Ecology - Mistletoe seed dispersal by a marsupial. *Nature* 408:929-930
- Audley-Charles MG (1991) Tectonics of the New-Guinea Area. *Annual Review of Earth and Planetary Sciences* 19:17-41
- Beehler BM, Pratt TK, Zimmerman DA (1986) *Birds of New Guinea*. Princeton University Press, Princeton, New Jersey
- Bennett ATD, Cuthill IC (1994) Ultraviolet vision in birds - What is its function? *Vision Research* 34:1471-1478
- Bennett ATD, Cuthill IC, Norris KJ (1994) Sexual selection and the mismeasure of color. *American Naturalist* 144:848-860
- Bennett ATD, Thery M (2007) Avian color vision and coloration: Multidisciplinary evolutionary biology. *American Naturalist* 169:S1-S6
- Ben-Tal Y, King RW (1997) Environmental factors involved in colouration of flowers of Kangaroo Paw. *Scientia Horticulturae* 72:35-48
- Berg CC (1989) Classification and Distribution of *Ficus*. *Experientia* 45:605-611

- Bertness MD, Leonard GH (1997) The role of positive interactions in communities: Lessons from intertidal habitats. *Ecology* 78:1976-1989
- Bertness MD, Leonard GH, Levine JM, Schmidt PR, Ingraham AO (1999) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. *Ecology* 80:2711-2726
- Bianconi GV, Mikich SB, Teixeira SD, Maia B (2007) Attraction of fruit-eating bats with essential oils of fruits: A potential tool for forest restoration. *Biotropica* 39:136-140
- Bluthgen N, Menzel F, Hovestadt T, Fiala B (2007) Specialization, constraints, and conflicting interests in mutualistic networks. *Current Biology* 17:341-346
- Bolmgren K, Eriksson O (2005) Fleshy fruits - origins, niche shifts, and diversification. *Oikos* 109:255-272
- Bonaccorso FJ (1998) Bats of Papua New Guinea. Conservation International, Washington, DC
- Bowmaker JK, Heath LA, Wilkie SE, Hunt DM (1997) Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Research* 37:2183-2194
- Bowmaker JK, Hunt DM (2006) Evolution of vertebrate visual pigments. *Current Biology* 16:R484-R489
- Bradshaw HD, Schemske DW (2003) Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* 426:176-178
- Bremer B, Eriksson O (1992) Evolution of Fruit Characteristics and Dispersal Modes in the Tropical Family Rubiaceae. *Biological Journal of the Linnean Society* 47:79-95
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution* 18:119-125
- Burnham KP, Anderson D (2003) Model selection and multi-model inference., 2nd edn. Springer, New York, NY
- Burns KC, Dalen JL (2002) Foliage color contrasts and adaptive fruit color variation in a bird-dispersed plant community. *Oikos* 96:463-469
- Cardoso da Silva JM, Tabarelli M (2000) Tree species impoverishment and the future flora of the Atlantic forest of Northeast Brazil. *Nature* 404:72-74
- Catoni C, Schaefer HM, Peters A (2008) Fruit for health: the effect of flavonoids on humoral immune response and food selection in a frugivorous bird. *Functional Ecology*. In press

- Chapman CA, Onderdonk DA (1998) Forests without primates: Primate/plant codependency. *American Journal of Primatology* 45:127-141
- Chittka L, Brockmann A (2005) Perception space - The final frontier. *Plos Biology* 3:564-568
- Chittka L, Raine NE (2006) Recognition of flowers by pollinators. *Current Opinion in Plant Biology* 9:428-435
- Cipollini ML, Bohs LA, Mink K, Paulk E, Böhning-Gaese K (2002) Secondary metabolites of ripe fleshy fruits: ecology and phylogeny in the genus *Solanum*. In: Levey DJ, Silva WR, Galetti M (eds) *Seed dispersal and Frugivory: Ecology, Evolution and Conservation*. CABI Publishing, Wallingford, pp 111-128
- Cipollini ML, Levey DJ (1991) Why some fruits are green when they are ripe - carbon balance in fleshy fruits. *Oecologia* 88:371-377
- Cipollini ML, Levey DJ (1997) Secondary metabolites of fleshy vertebrate-dispersed fruits: Adaptive hypotheses and implications for seed dispersal. *American Naturalist* 150:346-372
- Clark CJ, Poulsen JR, Levey DJ, Osenberg CW (2007) Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *American Naturalist* 170:128-142
- Clark CJ, Poulsen JR, Parker VT (2001) The role of arboreal seed dispersal groups on the seed rain of a lowland tropical forest. *Biotropica* 33:606-620
- Clark JS et al. (1999) Interpreting recruitment limitation in forests. *American Journal of Botany* 86:1-16
- Clark L (1991) Odor detection thresholds in tree swallows and Cedar Waxwings. *Auk* 108:177-180
- Clotfelter ED, Ardia DR, McGraw KJ (2007) Red fish, blue fish: trade-offs between pigmentation and immunity in *Betta splendens*. *Behavioral Ecology* 18:1139-1145
- Cordeiro NJ, Howe HF (2001) Low recruitment of trees dispersed by animals in African forest fragments. *Conservation Biology* 15:1733-1741
- Cordeiro NJ, Howe HF (2003) Forest fragmentation severs mutualism between seed dispersers and an endemic African tree. *Proceedings of the National Academy of Sciences of the United States of America* 100:14052-14056
- Croat TB (1978) *Flora of Barro Colorado Island*. Stanford University Press, Stanford, California

- Cuervo JJ, Shine R (2007) Hues of a dragon's belly: morphological correlates of ventral coloration in water dragons. *Journal of Zoology* 273:298-304
- Darwin C (1859) *On the origin of species by means of natural selection or the preservation of favoured races in the struggle of life*, London
- Das D, Wilkie SE, Hunt DM, Bowmaker JK (1999) Visual pigments and oil droplets in the retina of a passerine bird, the canary *Serinus canaria*: microspectrophotometry and opsin sequences. *Vision Research* 39:2801-2815
- de Ayala RM, Saino N, Moller AP, Anselmi C (2007) Mouth coloration of nestlings covaries with offspring quality and influences parental feeding behavior. *Behavioral Ecology* 18:526-534
- Dominy NJ (2004) Fruits, fingers, and fermentation: The sensory cues available to foraging primates. *Integrative and Comparative Biology* 44:295-303
- Dominy NJ, Lucas PW, Osorio D, Yamashita N (2001) The sensory ecology of primate food perception. *Evolutionary Anthropology* 10:171-186
- Dominy NJ, Svenning JC, Li WH (2003) Historical contingency in the evolution of primate color vision. *Journal of Human Evolution* 44:25-45
- Ducrocq S, Jaeger JJ, Sigé B (1993) Un mégachiroptère dans l'Eocène supérieur de Thaïlande: incidence dans la discussion phylogénique du groupe. *N. Jahrb. Geol. Paläotol. Mh.* 9:561-575
- Dumont ER (1999) The effect of food hardness on feeding behaviour in frugivorous bats (Phyllostomidae): an experimental study. *Journal of Zoology* 248:219-229
- Dumont ER, O'Neal R (2004) Food hardness and feeding behavior in old world fruit bats (Pteropodidae). *Journal of Mammalogy* 85:8-14
- Dumont ER, Weiblen GD, Winkelmann JR (2004) Preferences of fig wasps and fruit bats for figs of functionally dioecious *Ficus pungens*. *Journal of Tropical Ecology* 20:233-238
- Dyreson E, Maddison WP (2006) Rhetenor package for morphometrics In *Mesquite* 1.11
- Elangovan V, Marimuthu G, Kunz TH (2001) Temporal patterns of resource use by the short-nosed fruit bat, *Cynopterus sphinx* (Megachiroptera : Pteropodidae). *Journal of Mammalogy* 82:161-165
- Endler JA (1986) *Natural selection in the wild*. Princeton University Press, Princeton
- Endler JA (1990) On the Measurement and Classification of Color in Studies of Animal Color Patterns. *Biological Journal of the Linnean Society* 41:315-352

- Endler JA (1992) Signals, Signal Conditions, and the Direction of Evolution. *American Naturalist* 139:S125-S153
- Evans CS (2006) Accessory chemosignaling mechanisms in primates. *American Journal of Primatology* 68:525-544
- Felsenstein J (1985) Phylogenies and the Comparative Method. *American Naturalist* 125:1-15
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annual Review of Ecology Evolution and Systematics* 35:375-403
- Fischer KE, Chapman CA (1993) Frugivores and fruit syndromes - Differences in patterns at the genus and species level. *Oikos* 66:472-482
- Fleming TH, Breitwisch R, Whitesides GH (1987) Patterns of tropical vertebrate frugivore diversity. *Annual Review of Ecology and Systematics* 18:91-109
- Fragoso JMV, Silvius KM, Correa JA (2003) Long-distance seed dispersal by tapirs increases seed survival and aggregates tropical trees. *Ecology* 84:1998-2006
- Fuentes M (1995) How specialized are fruit-bird interactions? Overlap of frugivore assemblages within and between plant species. *Oikos* 74:324-330
- Gautier-Hion A et al. (1985) Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. *Oecologia* 65:324-337
- Giannini NP, Simmons NB (2003) A phylogeny of megachiropteran bats (Mammalia : Chiroptera : Pteropodidae) based on direct optimization analysis of one nuclear and four mitochondrial genes. *Cladistics-the International Journal of the Willi Hennig Society* 19:496-511
- Godinez-Alvarez H, Valiente-Banuet A, Rojas-Martinez A (2002) The role of seed dispersers in the population dynamics of the columnar cactus *Neobuxbaumia tetetzo*. *Ecology* 83:2617-2629
- Goff SA, Klee HJ (2006) Plant volatile compounds: Sensory cues for health and nutritional value? *Science* 311:815-819
- Gomulkiewicz R, Thompson JN, Holt RD, Nuismer SL, Hochberg ME (2000) Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist* 156:156-174
- Gould SJ, Vrba ES (1982) Exaptation - a Missing Term in the Science of Form. *Paleobiology* 8:4-15

- Grison-Pige L, Bessiere JM, Hossaert-McKey M (2002a) Specific attraction of fig-pollinating wasps: Role of volatile compounds released by tropical figs. *Journal of Chemical Ecology* 28:283-295
- Grison-Pige L, Hossaert-McKey M, Greeff JM, Bessiere JM (2002b) Fig volatile compounds - a first comparative study. *Phytochemistry* 61:61-71
- Gumbert A, Kunze J, Chittka L (1999) Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society of London Series B-Biological Sciences* 266:1711-1716
- Hall E (1997) Biological sequence alignment editor for Windows 95/98/NT. In <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- Halpern BS, Silliman BR, Olden JD, Bruno JP, Bertness MD (2007) Incorporating positive interactions in aquatic restoration and conservation. *Frontiers in Ecology and the Environment* 5:153-160
- Hampe A, Bairlein F (2000) Modified dispersal-related traits in disjunct populations of bird-dispersed *Frangula alnus* (Rhamnaceae): a result of its Quaternary distribution shifts? *Ecography* 23:603-613
- Harms KE, Wright SJ, Calderon O, Hernandez A, Herre EA (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature* 404:493-495
- Hart NS (2001) The visual ecology of avian photoreceptors. *Progress in Retinal and Eye Research* 20:675-703
- Harvey PH, Pagel MD (1991) *The comparative method in evolutionary biology*. Oxford University Press, Oxford
- Herrera CM (1985) Determinants of Plant-Animal Coevolution - the Case of Mutualistic Dispersal of Seeds by Vertebrates. *Oikos* 44:132-141
- Herrera CM (1987) Vertebrate-dispersed plants of the Iberian Peninsula - A study of fruit characteristics. *Ecological Monographs* 57:305-331
- Herrera CM (1989) Seed dispersal by animals: a role in angiosperm diversification? *American Naturalist* 133:309-322
- Herrera CM (1992) Interspecific variation in fruit shape: allometry, phylogeny, and adaptation to dispersal agents. *Ecology* 73:1832-1841
- Herrera CM (1998) Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: A 12-year study. *Ecological Monographs* 68:511-538

- Herrera CM (2002a) Correlated evolution of fruit and leaf size in bird-dispersed plants: species-level variance in fruit traits explained a bit further? *Oikos* 97:426-432
- Herrera CM (2002b) Seed dispersal by vertebrates. In: Herrera CM, Pellmyr O (eds) *Plant-Animal Interactions. An Evolutionary Approach*. Blackwell Science Ltd., Oxford, pp 185-208
- Herrera CM, Jordano P, Lopezsofia L, Amat JA (1994) Recruitment of a Mast-Fruiting, Bird-Dispersed Tree - Bridging Frugivore Activity and Seedling Establishment. *Ecological Monographs* 64:315-344
- Heymann EW (2006) The neglected sense-olfaction in primate behavior, ecology, and evolution. *American Journal of Primatology* 68:519-524
- Hill DS (1967) Figs (*Ficus* Spp) and Fig-Wasps (*Chalcidoidea*). *Journal of Natural History* 1:413-&
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42:182-192
- Hjerpe J, Hedenas H, Elmqvist T (2001) Tropical rain forest recovery from cyclone damage and fire in Samoa. *Biotropica* 33:249-259
- Hodgkison R et al. (2007) Chemical ecology of fruit bat foraging behavior in relation to the fruit odors of two species of paleotropical bat-dispersed figs (*Ficus hispida* and *Ficus scortechinii*). *Journal of Chemical Ecology* 33:2097-2110
- Howe HF (1986) Seed dispersal by fruit-eating birds and mammals. In: Murray D (ed) *Seed dispersal*. Academic Press, New York, pp 123-189
- Howe HF, Miriti MN (2000) No question: seed dispersal matters. *Trends in Ecology & Evolution* 15:434-436
- Howe HF, Miriti MN (2004) When seed dispersal matters. *Bioscience* 54:651-660
- Howe HF, Schupp EW, Westley LC (1985) Early Consequences of Seed Dispersal for a Neotropical Tree (*Virola-Surinamensis*). *Ecology* 66:781-791
- Hsiao C, Jacobs SWL, Chatterton NJ, Asay KH (1999) A molecular phylogeny of the grass family (*Poaceae*) based on the sequences of nuclear ribosomal DNA (ITS). *Australian Systematic Botany* 11:667-688
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754-755

- Hurtt GC, Pacala SW (1995) The Consequences of Recruitment Limitation - Reconciling Chance, History and Competitive Differences between Plants. *Journal of Theoretical Biology* 176:1-12
- Janson CH (1983) Adaptation of fruit morphology to dispersal agents in a Neotropical forest. *Science* 219:187-189
- Janzen DH (1970) Herbivores and Number of Tree Species in Tropical Forests. *American Naturalist* 104:501-&
- Janzen DH (1979) How to be a fig. *Annual Review of Ecology and Systematics* 10:13-51
- Janzen DH (1980) When is it coevolution? *Evolution* 34:611-612
- Janzen DH (1985) On ecological fitting. *Oikos* 45:308-310
- Janzen DH, Martin PS (1982) Neotropical Anachronisms - the Fruits the Gomphotheres Ate. *Science* 215:19-27
- Johnson SD, Steiner KE (2000) Generalization versus specialization in plant pollination systems. *Trends in Ecology & Evolution* 15:140-143
- Jordano P (1995) Angiosperm fleshy fruits and seed dispersers - A comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist* 145:163-191
- Jordano P (2000) Fruits and frugivory. In: Fenner M (ed) *Seeds. The Ecology of Regeneration in Plant Communities*. CABI Publishing, New York, pp 125-165
- Jousselin E, Rasplus JY, Kjellberg F (2003) Convergence and coevolution in a mutualism: Evidence from a molecular phylogeny of *Ficus*. *Evolution* 57:1255-1269
- Jurik TW (1983) Reproductive Effort and CO₂ Dynamics of Wild Strawberry Populations. *Ecology* 64:1329-1342
- Kalko EKV, Herre EA, Handley CO (1996) Relation of fig fruit characteristics to fruit-eating bats in the New and Old World tropics. *Journal of Biogeography* 23:565-576
- Kelber A, Vorobyev M, Osorio D (2003) Animal colour vision - behavioural tests and physiological concepts. *Biological Reviews* 78:81-118
- Knight RS, Siegfried WR (1983) Inter-relationships between type, size and colour of fruits and dispersal in Southern African trees. *Oecologia* 56:405-412
- Korine C, Kalko EKV (2005) Fruit detection and discrimination by small fruit-eating bats (Phyllostomidae): echolocation call design and olfaction. *Behavioral Ecology and Sociobiology* 59:12-23

- Korine C, Kalko EKV, Herre EA (2000) Fruit characteristics and factors affecting fruit removal in a Panamanian community of strangler figs. *Oecologia* 123:560-568
- Laman TG, Weiblen GD (1998) Figs of Gunung Palung National Park (West Kalimantan, Indonesia). *Tropical Biodiversity* 5:245-297
- Lambert FR (1992) Fig Dimorphism in Bird-Dispersed Gynodioecious Ficus. *Biotropica* 24:214-216
- Lambert FR, Marshall AG (1991) Keystone Characteristics of Bird-Dispersed Ficus in a Malaysian Lowland Rain-Forest. *Journal of Ecology* 79:793-809
- Laska M, Freist P, Krause S (2007) Which senses play a role in nonhuman primate food selection? A comparison between squirrel monkeys and spider monkeys. *American Journal of Primatology* 69:282-294
- Levey DJ (1987) Seed size and fruit-handling techniques of avian frugivores. *American Naturalist* 129:471-485
- Lomáscolo S, Speranza P, Kimball R (2008) Correlated evolution of fig size and color supports the Dispersal Syndromes hypothesis. *Oecologia* Published online DOI 10.1007/s00442-008-1023-0
- Lord JM (2004) Frugivore gape size and the evolution of fruit size and shape in southern hemisphere floras. *Austral Ecology* 29:430-436
- Lord JM, Markey AS, Marshall J (2002) Have frugivores influenced the evolution of fruit traits in New Zealand? In: Levey DJ, Silva WR, Galetti M (eds) *Seed dispersal and frugivory: Ecology, Evolution and Conservation*. CABI Publishing, Wallingford, pp 55-68
- Mack AL (1993) The sizes of vertebrate-dispersed fruits: a Neotropical-Paleotropical comparison. *American Naturalist* 142:840-856
- Mack AL (2000) Did fleshy fruit pulp evolve as a defence against seed loss rather than as a dispersal mechanism? *Journal of Biosciences* 25:93-97
- Maddison DR, Maddison WP (2000) *MacClade 4: Analysis of phylogeny and character evolution* In Sinauer Associates, Inc.
- Maddison DR, Maddison WP (2007) *Mesquite: A modular system for evolutionary analysis* In 2.01
- Maddison WP (1990) A method for testing the correlated evolution of two binary characters - Are gains or losses concentrated on certain branches of a phylogenetic tree? *Evolution* 44:539-557

- Mamlouk AM, Martinetz T (2004) On the dimensions of the olfactory perception space. *Neurocomputing* 58-60:1019-1025
- Mantel N (1967) Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Research* 27:209
- Márquez AL, Real R, Vargas JM (2004) Dependence of broad-scale geographical variation in fleshy-fruited plant species richness on disperser bird species richness. *Global Ecology and Biogeography* 13:295-304
- McKey DS (1975) The ecology of coevolved seed dispersal systems. In: Gilbert LE, Raven PH (eds) *Co-evolution of animals and plants*. University of Texas Press, Austin, pp 159-191
- Mikich SB, Bianconi GV, Helena B, Maia NS, Teixeira SD (2003) Attraction of the fruit-eating bat *Carollia perspicillata* to *Piper gaudichaudianum* essential oil. *Journal of Chemical Ecology* 29:2379-2383
- Moegenburg SM, Levey DJ (2002) Prospects for conserving biodiversity in Amazonian extractive reserves. *Ecology Letters* 5:320-324
- Muller-Landau HC, Wright SJ, Calderón O, Hubbell SP, Foster RB (2002) Assessing recruitment limitation: concepts, methods and case studies from a tropical forest. In: Levey DJ, Silva WR, Galetti M (eds) *Seed dispersal and frugivory: ecology, evolution and conservation*. CABI Publishing, Wallingford
- Murray KG, García-C JM (2002) Contributions of seed dispersal and demography to recruitment limitation in a Costa Rican cloud forest. In: Levey DJ, Silva WR, Galetti M (eds) *Seed dispersal and frugivory: ecology, evolution and conservation*. CABI Publishing, Wallingford
- Nathan R, Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution* 15:278-285
- Osorio D, Vorobyev M (1996) Colour vision as an adaptation to frugivory in primates. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263:593-599
- Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London Series B-Biological Sciences* 255:37-45
- Pagel M (1997) Inferring evolutionary processes from phylogenies. *Zoologica Scripta* 26:331-348
- Pagel M (1999) The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48:612-622

- Patel A, McKey D (1998) Sexual specialization in two tropical dioecious figs. *Oecologia* 115:391-400
- Peichl L (2005) Diversity of mammalian photoreceptor properties: Adaptations to habitat and lifestyle? *Anatomical Record Part a-Discoveries in Molecular Cellular and Evolutionary Biology* 287A:1001-1012
- Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14:817-818
- Poulsen JR, Clark CJ, Connor EF, Smith TB (2002) Differential resource use by primates and hornbills: Implications for seed dispersal. *Ecology* 83:228-240
- R Development Core Team (2008) R: A language and environment for statistical computing In R Foundation for Statistical Computing
- Redford KH (1992) The Empty Forest. *Bioscience* 42:412-422
- Reekie EG, Bazzaz FA (1987) Reproductive Effort in Plants .1. Carbon Allocation to Reproduction. *American Naturalist* 129:876-896
- Regan BC, Julliot C, Simmen B, Vienot F, Charles-Dominique P, Mollon JD (2001) Fruits, foliage and the evolution of primate colour vision. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 356:229-283
- Reid N (1991) Coevolution of Mistletoes and Frugivorous Birds. *Australian Journal of Ecology* 16:457-469
- Rey PJ, Alcantara JM (2000) Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. *Journal of Ecology* 88:622-633
- Rey PJ, Gutierrez JE, Alcantara J, Valera F (1997) Fruit size in wild olives: implications for avian seed dispersal. *Functional Ecology* 11:611-618
- Rey PJ et al. (2006) The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology* 19:21-34
- Rice WR (1989) Analyzing Tables of Statistical Tests. *Evolution* 43:223-225
- Ridley HN (1930) The dispersal of plants throughout the world. L. Reeve & Co., Ltd., Ashford
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574

- Rønsted N, Weiblen GD, Cook JM, Salamin N, Machado CA, Savolainen V (2005) 60 million years of co-divergence in the fig-wasp symbiosis. *Proceedings of the Royal Society B-Biological Sciences* 272:2593-2599
- Schaefer HM, Levey DJ, Schaefer V, Avery ML (2006) The role of chromatic and achromatic signals for fruit detection by birds. *Behavioral Ecology* 17:784-789
- Schaefer HM, McGraw KJ, Catoni C (2008) Birds use fruit colour as honest signal of dietary antioxidant rewards. *Functional Ecology* In press
- Schaefer HM, Schaefer V (2007) The evolution of visual fruit signals: concepts and constraints. In: Dennis AJ, Schupp EW, Green RJ, Wescott DA (eds) *Seed dispersal: Theory and its application in a changing world*. CABI, Wallingford, UK, pp 59-77
- Schaefer HM, Schaefer V, Levey DJ (2004) How plant-animal interactions signal new insights in communication. *Trends in Ecology & Evolution* 19:577-584
- Schaefer HM, Schaefer V, Vorobyev M (2007) Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *American Naturalist* 169:S159-S169
- Schaefer HM, Schmidt V (2004) Detectability and content as opposing signal characteristics in fruits. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271:S370-S373
- Schlick-Steiner BC et al. (2006) A multidisciplinary approach reveals cryptic diversity in Western Palearctic Tetramorium ants (Hymenoptera : Formicidae). *Molecular Phylogenetics and Evolution* 40:259-273
- Schmidt V, Schaefer HM, Winkler H (2004a) Conspicuousness, not colour as foraging cue in plant-animal signaling. *Oikos* 106:551-557
- Schmidt V, Schaefer HM, Winkler H (2004b) Conspicuousness, not colour as foraging cue in plant-animal signalling. *Oikos* 106:551-557
- Schupp EW (1988) Seed and Early Seedling Predation in the Forest Understory and in Treefall Gaps. *Oikos* 51:71-78
- Schupp EW (1993) Quantity, Quality and the Effectiveness of Seed Dispersal by Animals. *Vegetatio* 108:15-29
- Schupp EW, Fuentes M (1995) Spatial Patterns of Seed Dispersal and the Unification of Plant-Population Ecology. *Ecoscience* 2:267-275
- Shanahan M (2000) *Ficus* seed dispersal guilds: ecology, evolution and conservation implications. In. University of Leeds, UK

- Shanahan M, Compton SG (2001) Vertical stratification of figs and fig-eaters in a Bornean lowland rain forest: how is the canopy different? *Plant Ecology* 153:121-132
- Shanahan M, Harrison RD, Yamuna R, Boen W, Thornton IWB (2001a) Colonization of an island volcano, Long Island, Papua New Guinea, and an emergent island, Motmot, in its caldera lake. V. Colonization by figs (*Ficus* spp.), their dispersers and pollinators. *Journal of Biogeography* 28:1365-1377
- Shanahan M, So S, Compton SG, Corlett R (2001b) Fig-eating by vertebrate frugivores: a global review. *Biological Reviews* 76:529-572
- Smith CI, Pellmyr O, Althoff DM, Balcazar-Lara M, Leebens-Mack J, Segraves KA (2008) Pattern and timing of diversification in *Yucca* (Agavaceae): specialized pollination does not escalate rates of diversification. *Proceedings of the Royal Society B-Biological Sciences* 275:249-258
- Spiegel O, Nathan R (2007) Incorporating dispersal distance into the disperser effectiveness framework: frugivorous birds provide complementary dispersal to plants in a patchy environment. *Ecology Letters* 10:718-728
- Strauss SY, Lau JA, Carroll SP (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters* 9:354-371
- Sumner P, Mollon JD (2000a) Catarrhine photopigments are optimized for detecting targets against a foliage background. *Journal of Experimental Biology* 203:1963-1986
- Sumner P, Mollon JD (2000b) Chromaticity as a signal of ripeness in fruits taken by primates. *Journal of Experimental Biology* 203:1987-2000
- SurrIDGE AK, Osorio D, Mundy NI (2003) Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution* 18:198-205
- Swofford DL (2003) PAUP*: Phylogenetics analysis using parsimony (*and other methods), version 4.0 In Sinauer
- Tamboia T, Cipollini ML, Levey DJ (1996) An evaluation of vertebrate seed dispersal syndromes in four species of black nightshade (*Solanum* sect *Solanum*). *Oecologia* 107:522-532
- Temeles EJ, Kress WJ (2003) Adaptation in a plant-hummingbird association. *Science* 300:630-633
- Terborgh J (1986) Keystone plant resources in the tropical forest. In: Soule ME (ed) *Conservation Biology: the science of scarcity and diversity*. Sinauer Associates, Massachusetts, pp 330-344

- Terborgh J, Pitman N, Silman M, Schlichter H, Núñez P (2002) Maintenance of tree diversity in tropical forests. In: D. J. Levey WRS, and M. Galetti (ed) Seed dispersal and frugivory: Ecology, Evolution and Conservation. CABI Publishing, Wallingford, pp 1-17
- Thies W, Kalko EKV, Schnitzler HU (1998) The roles of echolocation and olfaction in two Neotropical fruit-eating bats, *Carollia perspicillata* and *C. castanea*, feeding on *Piper*. *Behavioral Ecology and Sociobiology* 42:397-409
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic acids research* 22:4673-4680
- Thompson JN (1994) *The coevolutionary process*. The University of Chicago Press
- Thompson JN (1999) Specific hypotheses on the geographic mosaic of coevolution. *American Naturalist* 153:S1-S14
- Thompson JN, Pellmyr O (1992) Mutualism with Pollinating Seed Parasites Amid Co-Pollinators - Constraints on Specialization. *Ecology* 73:1780-1791
- Thornton IWB, Compton SG, Wilson CN (1996) The role of animals in the colonization of the Krakatau Islands by fig trees (*Ficus* species). *Journal of Biogeography* 23:577-592
- Titus JH, Holbrook NM, Putz FE (1990) Seed-Germination and Seedling Distribution of *Ficus-Pertusa* and *F-Tuerckheimii* - Are Strangler Figs Autotoxic. *Biotropica* 22:425-428
- Traveset A, Willson MF, Verdu M (2004) Characteristics of fleshy fruits in southeast Alaska: phylogenetic comparison with fruits from Illinois. *Ecography* 27:41-48
- Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88:225-238
- van der Pijl L (1961) *Ecological Aspects of Flower Evolution* .2. Zoophilous Flower Classes. *Evolution* 15:44-&
- van der Pijl L (1969) *Principles of dispersal of higher plants*. Academic Press, Orlando, Fla.
- Vázquez DP (2005) Degree distribution in plant-animal mutualistic networks: forbidden links or random interactions? *Oikos* 108:421-426
- Vázquez DP, Aizen MA (2004) Asymmetric specialization: A pervasive feature of plant-pollinator interactions. *Ecology* 85:1251-1257
- Voigt FA, Bleher B, Fietz J, Ganzhorn JU, Schwab D, Bohning-Gaese K (2004) A comparison of morphological and chemical fruit traits between two sites with different frugivore assemblages. *Oecologia* 141:94-104

- Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society of London Series B-Biological Sciences* 265:351-358
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology* 77:1043-1060
- Weiblen GD (2000) Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. *American Journal of Botany* 87:1342-1357
- Weiblen GD (2002) How to be a fig wasp. *Annual Review of Entomology* 47:299-330
- Westoby M, Leishman MR, Lord JM (1995) On misinterpreting the 'phylogenetic correction'. *Journal of Ecology* 83:531-534
- Wheelwright NT (1985) Fruit size, gape width, and the diets of fruit-eating birds. *Ecology* 66:808-818
- Wheelwright NT, Janson CH (1985) Colors of fruit displays of bird-dispersed plants in two tropical forests. *American Naturalist* 126:777-799
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand D, Sminsky J, White T (eds) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp 315-322
- Whittaker RJ, Jones SH (1994) The role of frugivorous bats and birds in the rebuilding of a tropical forest ecosystem, Krakatau, Indonesia. *Journal of Biogeography* 21:245-258
- Wiebes JT (1979) Co-Evolution of Figs and Their Insect Pollinators. *Annual Review of Ecology and Systematics* 10:1-12
- Willson MF, Irvine AK, Walsh NG (1989) Vertebrate Dispersal Syndromes in Some Australian and New-Zealand Plant-Communities, with Geographic Comparisons. *Biotropica* 21:133-147
- Willson MF, Whelan CJ (1990) The evolution of fruit color in fleshy-fruited plants. *American Naturalist* 136:790-809
- Wysocki CJ, Preti G (2004) Facts, fallacies, fears, and frustrations with human pheromones. *Anatomical Record Part a-Discoveries in Molecular Cellular and Evolutionary Biology* 281A:1201-1211
- Zar JH (1999) *Biostatistical Analysis*, 4th edn. Prentice-Hall, New Jersey

Zerega NJC, Clement WL, Datwyler SL, Weiblen GD (2005) Biogeography and divergence times in the mulberry family (Moraceae). *Molecular Phylogenetics and Evolution* 37:402-416

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. In: www.bio.utexas.edu/faculty/antisense/garli/Garli.html. University of Texas, Austin.

BIOGRAPHICAL SKETCH

I completed my undergraduate studies at the Universidad Nacional de Tucumán (UNT), in Argentina, and obtained the title of Licenciata in Biological Sciences, with an orientation in Zoology in 1998. I obtained various honors for graduating with the highest average in the class of 1998 for all students in the College of Natural Sciences, namely, being the carrier of the National flag in official events, three medals given by the Department of Zoology, the College of Natural Sciences, and the Graduate School, and honorary diplomas by the Biological Sciences Graduate Association and the Argentinean Federation of University Women.

I obtained a graduate fellowship from the Secretariat of Science and Technology at the UNT to complete my graduate studies there. I started a project on the importance of birds and bats for the dispersal of a local species, *Solanum riparium* (Solanaceae) but, shortly after I also obtained a Fulbright Fellowship to go to graduate school in the United States. I pursued that opportunity and came to the University of Florida, where I enrolled in the MS and then Ph.D. programs in the Department of Zoology in August of 2000.

I obtained several grants totaling approximately \$35,000 to complete my field work in Papua New Guinea. Funding was provided by NSF, Rufford Foundation, Garden Club of America, Lube Foundation, Sigma Xi (two Grants in Aid for Research), American Women in Science, Sophie Danforth Foundation, among others. I also obtained several fellowships to support myself while taking courses and writing my dissertation, such as the Fulbright Fellowship mentioned above, an International Women Fellowship from P.E.O., and a Dissertation Fellowship from CLAS. I was also credited for “outstanding academic achievement” by an International Student Award by the University of Florida.