VARIAÇÃO MORFOLÓGICA E QUÍMICA DOS FRUTOS NA ESCOLHA DOS ANIMAIS FRUGÍVOROS DA MATA ATLÂNTICA

ELIANA CAZETTA

Tese apresentada ao Instituto de Biociências da Universidade Estadual Paulista "Julio de Mesquita Filho", Campus de Rio Claro, para a obtenção do título de Doutor em Ciências Biológicas (Área de Concentração: Biologia Vegetal)

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Dedico esta tese aos meus pais
Luiz e Nice

À querida Lu, irmã que a vida me deu e
melhor amiga que eu poderia escolher.

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RESUMO

As características dos frutos como morfologia e química têm sido tradicionalmente explicadas como resultado da seleção dos dispersores de sementes. Mas a importância dos frugívoros em moldar as características dos frutos tem sido recentemente questionada sob diferentes perspectivas. Alguns estudos sugerem que outras interações podem simultaneamente atuar sobre a evolução das características dos frutos. Muitos organismos atuam como predadores de sementes e seus efeitos precisam ser considerados. Desta maneira, as plantas enfrentam um dilema evolutivo entre atração aos legítimos dispersores de sementes e defesa contra predadores e patógenos. Neste estudo, primeiramente nós avaliamos as características dos frutos relacionadas com atração e defesa em uma ampla amostra de frutos da Mata Atlântica, da Ilha do Cardoso, São Paulo, Brasil. Posteriormente, nós testamos como algumas características específicas influenciam o consumo pelas aves frugívoras. Nós observamos que as características morfológicas e químicas dos frutos apresentam sinal filogenético e em geral padrões independentes de co-variação. Ao contrário, as cores e os contrates dos frutos não apresentam sinal filogenético e frutos mais saturados são ricos em lipídeos e energia mas pobres em carboidratos. Portanto, nós sugerimos que o grau de saturação da cor pode indicar a qualidade nutricional dos frutos. As aves detectaram consistentemente frutos com maior contraste cromático do que o contraste acromático. Além disso, as aves frugívoras selecionam frutos ricos em lipídeos e energia e pobres em compostos secundários. Nós concluímos que a preferência das aves por determinadas características dos frutos não afeta necessariamente a evolução das mesmas, uma vez que, para isso ocorrer é necessário que as aves selecionem entre indivíduos da mesma espécie e não entre espécies diferentes. Por essa razão, no nosso

estudo observamos que as características dos frutos são pouco associadas entre si, com exceção da cor, o que pode indicar uma adaptação aos animais frugívoros.

ABSTRACT

Fruit characteristics such as morphology and chemistry have traditionally been explained as the result of adaptations to their seed dispersers. But the importance of frugivores in shaping fruit traits has now been questioned from a number of perspectives. Some studies suggested that other interactions simultaneously shape the evolution of fruit traits. Many organisms act as seed predators and their effects must be taken into account. Therefore, plants are faced with an evolutionary dilemma between attraction to legitimate seed dispersers and defense against seed predators and pathogens. In this study we focused on fruit traits related to attractiveness and defense. We first evaluated how fruit characteristics interact in a broad sample of Atlantic rainforest species, at Cardoso Island, São Paulo, Brazil, after accounting for phylogeny. We then evaluated specific fruit characteristics to test whether they influence consumption by birds. We found that morphological and nutritional traits showed phylogenetic signal and in general independent patterns of covariation. On the contrary, fruit color and contrast did not present phylogenetic signal and saturated fruits are rich in energy and lipids, and poor in carbohydrates. Thus we suggest that saturated fruits may indicate fruit quality. Birds consistently detected fruits with higher chromatic contrasts rather than achromatic ones. Frugivorous birds also selected lipid and energy-rich fruits and fruits low defended by secondary compounds. We conclude that in spite of bird's preferences these processes do not operate on the evolution of fruit traits, since this requires birds to differentiate between plant individual of the same and not different species. Therefore, in our study we observed that fruit traits are in general weak associate, with the exception of fruit color, that may indicate fruit adaptation to frugivores.

INTRODUÇÃO GERAL

Estudos sobre as interações entre plantas e frugívoros originalmente basearam-se na literatura emergente de polinização e herbivoria nas décadas de 70 e 80, e desenvolveram um paradigma central sobre co-evolução entre frutos e dispersores de sementes. Desde então, as características dos frutos carnosos têm sido identificadas e interpretadas como adaptações aos dispersores de sementes (Howe 1986, Fleming *et al.* 1993). Teoricamente, diferenças previsíveis na qualidade de dispersão entre os diferentes tipos de frugívoros podem influenciar as características dos frutos, e de fato, muitos estudos demonstraram exemplos desta influência (Janson 1983, Gautier-Hion *et al.* 1985, Johnson *et al.* 1985, Willson *et al.* 1989, Gautier-Hion 1990, Schupp 1992).

No entanto, estudos recentes têm questionado esta visão, por terem identificado limitações para a co-evolução entre plantas e dispersores de sementes. Quando as características dos frutos como tamanho, forma e conteúdo nutricional são examinadas utilizando modelos filogenéticos rigorosos, estas características são melhor explicadas pela filogenia e não por requerimentos dos dispersores de sementes (Herrera 1987, 1992, 1998, Jordano 1995, Bremer & Eriksson 1992). Além disso, outros obstáculos foram identificados para a co-evolução estreita entre plantas e frugívoros, como, limitações genéticas (Howe 1984) e inconsistência nas pressões seletivas ao longo do tempo e do espaço (Herrera 1998, Thompson 2005). Isso não implica que os frugívoros não exerçam pressões adaptativas nos frutos, mas sugere que os padrões e as síndromes que pareciam obviamente ser resultado de co-evolução, não são tão simples como pensados previamente (Jordano 1995).

Talvez uma das principais razões para as conclusões confusas sobre as interações entre dispersores de sementes e plantas é que a maioria dos estudos prévios tem ignorado o papel dos compostos secundários na composição química dos frutos (Cipollini & Levey 1997a). Uma grande variedade de organismos interage simultaneamente com as plantas e os dispersores de sementes, incluindo potenciais agentes que também selecionam características dos frutos e desta maneira afetam a interação das plantas com os dispersores. Entre esses organismos podemos citar os frugívoros antagonistas que consomem os frutos mas não dispersam as sementes. Existe uma grande diversidade de animais que atuam como predadores de sementes (Hulme & Benkman 2002). Entre os frugívoros antagonistas melhor estudados estão algumas espécies de aves e mamíferos predadores de sementes. No entanto, invertebrados e patógenos (como fungos e bactérias), que frequentemente são ignorados nos estudos de dispersão, exercem uma grande influência nas características dos frutos que também estão relacionadas com a dispersão de sementes (Herrera 1986).

O ponto crítico é que além do próprio declínio no sucesso reprodutivo, a destruição dos frutos por invertebrados e patógenos também tem uma importância evolutiva no processo de dispersão de sementes, porque a seleção dos frutos pelos frugívoros antagonistas é geralmente dependente das características dos frutos, o que consequentemente influencia as preferências ou comportamento dos dispersores de sementes (Herrera 1986). Desta maneira, os frutos, os dispersores de sementes e os frugívoros antagonistas podem ser vistos como componentes de uma tríade evolutiva onde cada parte interage simultaneamente com as outras partes (Herrera 1984, Bulchholz and Levey 1990) (Figura 1). Portanto, os frutos sofrem um conflito evolutivo entre atratividade aos dispersores de sementes e defesa contra patógenos e predadores

(Cipollini & Levey 1997b). Os compostos secundários presentes na polpa dos frutos maduros são provavelmente os mediadores desse conflito (Janzen 1977, Herrera 1982, Cipollini & Stiles 1993).

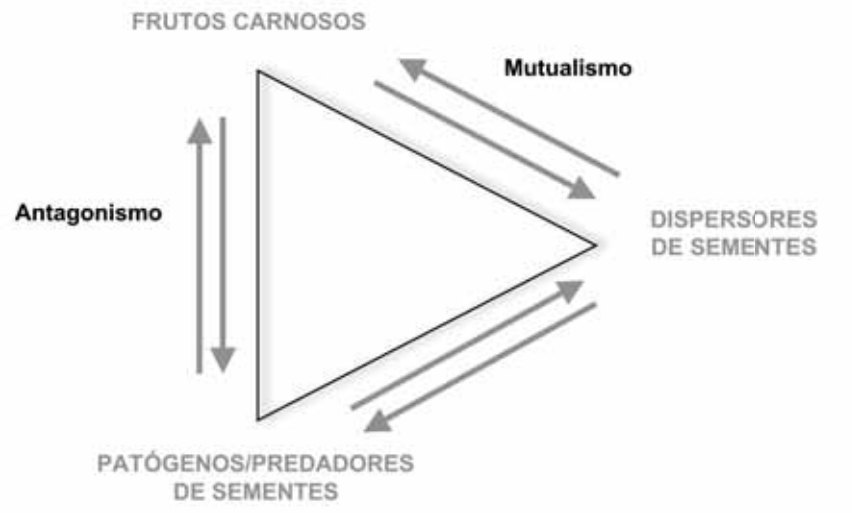

Figura 1) Ilustração da tríade evolutiva entre frutos carnosos, dispersores de sementes e frugívoros antagonistas.

Cipollini *et al.* (2002) foram os primeiros a utilizar controles filogenéticos ao examinar padrões de variação na morfologia, conteúdo nutricional e compostos secundários, utilizando várias espécies do gênero *Solanum*. Izhaki *et al.* (2002) avaliaram uma escala ainda mais refinada ao estudar a variação nas características químicas e morfológicas entre indivíduos de *Rhamnus alaternus*. Esses autores observaram que indivíduos com alta disponibilidade de água para os frutos apresentam maiores concentrações de carboidratos não estruturais e também são mais protegidos por altas concentrações de emodin (um composto secundário). Sendo assim, é difícil

considerar separadamente as hipóteses sobre as relações entre compostos secundários, frugívoros antagonistas e dispersores de sementes.

Uma série de hipóteses foi proposta para explicar o valor adaptativo dos compostos secundários presentes na polpa de frutos carnosos (Cipollini & Levey 1997a). Ente elas, apenas uma considera explicitamente o papel dos frugívoros antagonistas para explicar a presença dos compostos secundário nos frutos maduros. A hipótese do balanço entre atração e defesa (*defense trade-off hypothesis*) presume que os compostos secundários representam um balanço entre defesa contra os frugívoros antagonistas e atração aos dispersores de sementes (Cipollini & Levey 1997a). Esta hipótese gerou dois modelos que levam a predições contraditórias sobre o conteúdo de nutrientes e compostos secundários nos frutos. O primeiro modelo, o da taxa de remoção (*removal rate model*), sugere que os frutos ricos são rapidamente removidos e dessa maneira requerem um menor investimento em defesa, levando assim a uma relação negativa entre conteúdo nutricional e compostos secundários. Por outro lado, a hipótese da titulação nutriente/toxina (*nutrient/toxin titration model*) prevê uma relação positiva entre conteúdo nutricional e compostos de defesa, onde os frutos ricos são suficientemente palatáveis para garantirem altos níveis de defesa (Cipollini & Levey 1997a).

Cor dos frutos

Assim como as outras características dos frutos carnosos, tradicionalmente a cor dos frutos tem sido considerada uma adaptação aos animais frugívoros (Darwin 1862, Kerner 1895, Willson & Whelan 1990). Esta hipótese adaptativa recebeu suporte recentemente em um estudo interespecífico comparativo, onde foi constatado que

limitações filogenéticas são menores nas cores dos frutos do que em outras características (Voigt *et al.* 2004). Durante a década de 80 e início da década de 90, as hipóteses sobre a influência dos dispersores de sementes na evolução das cores dos frutos presumiam que esses tinham marcadas preferências por certas cores. Embora alguns estudos tenham reportado preferência por cor em algumas espécies de animais frugívoros (Puckey *et al.* 1996, Siitari *et al.* 1999, Whitney 2005), essas preferências são perdidas com a experiência (Schmidt & Schaefer 2004) e a maioria dos estudos não corrobora a hipótese da preferência devido a inconsistência na seleção de cores (Willson *et al.* 1990, Willson & Comet 1993, Traeset & Willson 1998, Schmidt *et al.* 2004).

Por outro lado, os dispersores de sementes podem selecionar as cores dos frutos por consistentemente detectarem essa cor mais facilmente (Kerner 1895). Essa hipótese sempre foi negligenciada devido a dificuldade em avaliar a conspicuidade das cores de acordo com a percepção dos animais. Portanto, a maioria dos estudos categorizou as cores dos frutos de acordo com a visão humana, o que é inadequado para outros grupos de consumidores, como as aves por exemplo, que apresentam uma sensibilidade espectral e amplitude de percepção diferentes da humana (Bennett *et al.* 1994). Atualmente, pequenos espectrômetros permitem a quantificação das cores que é independente das propriedades dos sistemas visuais e desta forma aplicável a diferentes grupos. Baseado em modelos de evolução dos sinais visuais, Schluter & Price (1993) documentaram que o conteúdo e a detectabilidade de um sinal são os dois fatores que dirigem a evolução dos sinais. Uma das predições chave deste modelo, que os sinais evoluíram para aumentar a detectabilidade, tem recentemente sido aplicada ao contexto da evolução dos sinais na comunicação entre animais e plantas. Há muito tempo tem sido reconhecido que frutos vermelhos e pretos são os mais comuns em ambientes

temperados e tropicais (Wheelwright & Janson 1985, mas ver Lord *et al.* 2002). Consistente com a teoria dos sinais, de que estes evoluíram para aumentar a detectabilidade, dois estudos documentaram que frutos vermelhos e pretos são os que apresentam os maiores contrastes com fundos típicos como o proporcionado pelas folhas (Lee *et al.* 1994, Schmidt *et al.* 2004).

A evolução das cores dos frutos como sinal é, no entanto, muito mais complexa do que abordado nestes estudos. Em uma floresta tropical na Venezuela os frutos apresentaram um padrão de sinais dicotômico. Os frutos vermelhos e pretos foram os mais conspícuos, mas não indicaram o conteúdo nutricional, enquanto outras cores que são menos conspícuas, sinalizaram o conteúdo de proteínas, carboidratos e taninos (Schaefer & Schmidt 2004).

Para entender a evolução das cores dos frutos como sinal é necessário a integração de informações sobre outras variáveis dos frutos, como morfologia, química e compostos secundários. Além disso, somente avaliando todas essas características, com controle filogenético rigoroso, será possível avaliar propriamente a relação entre plantas e dispersores de sementes. Desta forma, o objetivo geral deste estudo foi avaliar como as características dos frutos estão relacionadas e como essas características influenciam a escolha pelos animais frugívoros.

Objetivos específicos

Capítulo 1) As características fenotípicas dos frutos quando examinadas por modelos filogenéticos rigorosos, são melhor explicadas pela filogenia e não por requerimentos dos dispersores de sementes. Sendo assim, o capítulo 1 teve como

objetivo principal avaliar como a filogenia influencia as características de atração e defesa em frutos carnosos. Os objetivos específicos foram:

1. Avaliar se as características fenotípicas dos frutos carnosos apresentam sinal filogenético e quantificar a quantidade de sinal para cada variável.

2. Avaliar como as características fenotípicas estão correlacionadas depois de considerar a filogenia. As predições foram:

a. Frutos pequenos apresentam cores mais contrastantes do que frutos grandes.

b. Frutos menos contrastantes apresentam maiores concentrações de compostos secundários e menores quantidades de nutrientes.

Capítulo 2) Considerando as cores dos frutos como sinal e tendo em vista que a detectabilidade de um sinal visual é determinada pelo seu contraste contra um fundo, este capítulo teve como objetivo:

1. Avaliar a importância relativa dos contrastes cromáticos e acromáticos para a detecção dos frutos por aves frugívoras.

Capítulo 3) A hipótese do balanço entre atração e defesa é a mais aceita para explicar a presença de compostos secundários em frutos carnosos. Esse capítulo teve como objetivo principal avaliar como a composição química dos frutos influencia o consumo dos dispersores e patógenos, testando a hipótese do balanço entre atração e defesa e seus modelos. Os objetivos específicos foram:

1. Avaliar se os frutos ricos são mais atrativos para patógenos e vertebrados dispersores de sementes

2. Determinar se os frutos mais protegidos por compostos secundários persistem por maiores períodos, como sugerido pela hipótese do balanço entre atração e defesa.
3. Verificar qual dos modelos alternativos, o da taxa de remoção ou da titulação nutriente/toxina, melhor explicam o conteúdo nutricional dos frutos carnosos.

ÁREA DE ESTUDO

Ilha do Cardoso

A Ilha do Cardoso (hoje Parque Estadual da Ilha do Cardoso - PEIC) é uma unidade de conservação de uso indireto, criada em 1962 por estar situada no terceiro maior estuário do mundo, o complexo estuarino-lagunar de Iguape-Cananéia-Paranaguá, localizado no litoral sul do Estado de São Paulo (Figura 2). O parque abrange uma área de aproximadamente 15.100 ha (48°05'42" W, 25°03'05" S e 48°53'48" W, 25°18'18" S), separado do continente pelo canal de Trapandé.

O clima da ilha é influenciado por fatores locais como maritimidade, topografia acidentada e vegetação, sendo do tipo megatérmico superúmido, sem estação seca definida e com grande excesso de chuvas no verão, alcançando índices anuais de 3.000 mm (Funari *et al.* 1987). Segundo Gutjahr (1993), a precipitação anual estimada no período de 1976 a 1985 apresentou mínima de aproximadamente 1400 mm, média de 2000 mm e máxima de 2500 a 3000 mm. Pfeifer (1981, 1982), por meio de dados meteorológicos obtidos na cidade de Cananéia, estima que no período de 1949 a 1965 as médias das temperaturas máximas foram de 25°C e das mínimas 17,4°C, a temperatura máxima absoluta foi de 32,4°C e a mínima absoluta de 13°C. Já Funari *et al*. (1987), baseados em dados de 1956 a 1975, também obtidos na cidade de Cananéia, estimaram uma temperatura média anual de 21,2°C.

Figura 2) Localização da Ilha do Cardoso no Estado de São Paulo e imagem detalhada da área de estudo (A – núcleo Perequê, onde foi realizado o presente estudo) Cananéia, São Paulo, Brasil.

A topografia é predominantemente montanhosa, com a região central dominada por elevações que atingem 800 m. A vegetação da Ilha do Cardoso é formada exclusivamente por Floresta Atlântica. Um estudo detalhado realizado pelo Instituto de Botânica (CINP-SMA) registrou 986 espécies vegetais em 483 gêneros e 143 famílias. As famílias mais representativas são: Orchidaceae (118 espécies), Myrtaceae (70 espécies), Leguminosae (63 espécies), Poaceae (57 espécies), Rubiaceae (50 espécies), Compositae (43 espécies) e Bromeliaceae (41 espécies) (Barros *et al.* 1991). Ocorrem cinco formações vegetais naturais na área de estudo: vegetação de mangue, vegetação pioneira de dunas, vegetação de restinga arbórea e arbustiva, floresta tropical de planície litorânea e floresta pluvial tropical de encosta (Noffs & Baptista Noffs 1982). O presente trabalho foi realizado na vegetação de restinga arbórea e arbustiva, floresta tropical de planície litorânea e floresta pluvial tropical de encosta (Figura 3 e 4).

Figura 3) Gradiente vegetacional da Ilha do Cardoso, destacando as três formações florestais utilizadas neste estudo.

Caracterização da Vegetação

Vegetação de Mangue

A vegetação de mangue cobre os sedimentos finos e lodosos da planície litorânea encontrados na foz dos rios e ao longo do canal de Ararapira, constituindo os manguezais. Segundo Schaeffer-Novelli (1987) os mangues da Ilha do Cardoso apresentam padrão muito semelhante aos demais mangues da costa brasileira. Em termos ecológicos, são caracterizados pela presença de substrato constituído por sedimentos não consolidados, permanentemente inundado, pobre em oxigênio e rico em matéria orgânica. As espécies vegetais adaptadas a essas condições extremas são poucas: *Rhizophora mangle* (Rhizophoraceae), *Laguncularia racenos*a (Combretaceae) e *Avicennia schaueriana* (Verbenaceae). Nas bordas dos manguezais por sua vez ocorrem comumente *Hibiscus pernambucensis* (Malvaceae) e *Spartina ciliata* (Poaceae).

Vegetação Pioneira de Dunas

A vegetação que fixa as elevações de areia nas praias litorâneas brasileiras são geralmente denominadas vegetação pioneira de dunas. As plantas que caracterizam essa vegetação são psamófilas e halófitas, caracterizadas por serem estoloníferas e rizomatosas (Barros *et al.* 1991). Estas espécies são adaptadas a insolação e salinidade. Entre as espécies mais comum, encontram-se *Spartina ciliata* (Poaceae), *Ipomea pés-caprea* (Convolvulaceae), *Hydrocotyle bonariensis* (Umbelliferae), *Acicarpha sathulata* (Calyceraceae), *Diodia teres* e *Diodia radula* (Rubiaceae).

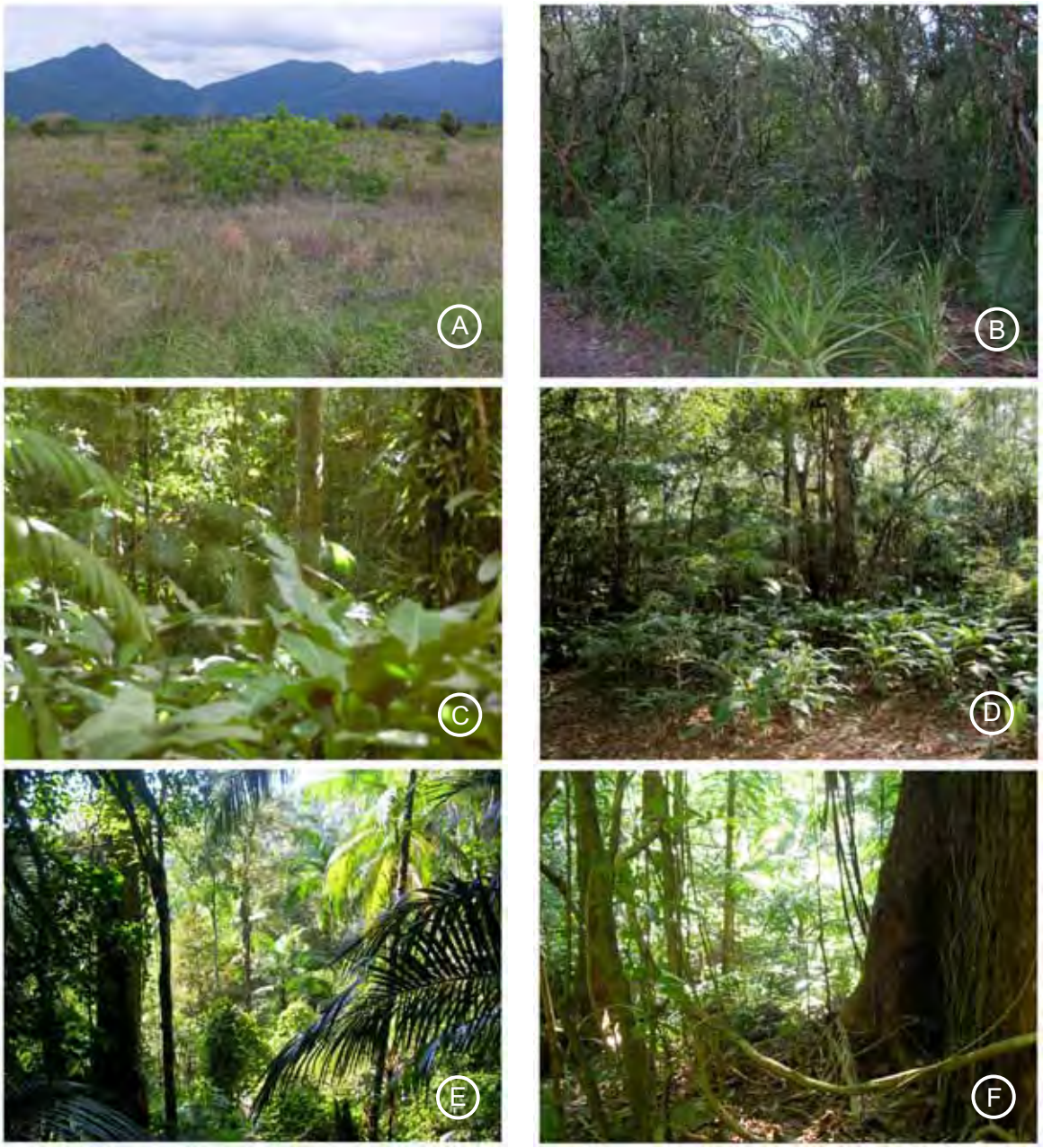

Figura 4) Formações vegetais utilizadas neste estudo no PEIC: A) Restinga arbustiva; B) Restinga arbórea; C e D) Floresta Tropical de Planície Litorânea; E e F) Floresta Pluvial Tropical de Encosta.

Vegetação de Restinga

A vegetação de restinga pode ser considerada como o conjunto de comunidades fisionomicamente distintas sob influência marinha e fluvio marinha, distribuídas em mosaico e que ocorrem como comunidades edáficas por dependerem mais da natureza do solo do que do clima (Sugyama 1998). Essa vegetação cobre a maior parte da planície arenosa da Ilha do Cardoso e é caracterizada por um complexo de diferentes comunidades vegetais que se interpenetram (De Grande e Lopes 1981).

A restinga do Pereirinha (núcleo Perequê – onde este estudo foi realizado) é caracterizada por uma vegetação predominantemente florestal, incluindo formações arbóreas abertas e baixas, com 4-5 metros de altura, até formações arbóreas fechadas, com até 15 metros de altura (Sugyama 1998). Barros *et al.* (1991), descreveram uma formação mais arbustiva na porção baixa do pós-praia dominada por *Dalbergia ecastaphyllum*, *Sophora tomentosa* e *Tibouchina holosericea*. Trata-se de uma vegetação baixa, com indivíduos espaçados ou não entre si, sob um substrato arenoso.

As áreas de restinga mais aberta são bem iluminadas apresentando árvores com altura média de 5 metros, bem ramificadas a partir da base, com um estrato herbáceo bastante conspícuo, onde predominam pteridófitas e bromélias. O solo é arenoso com uma fina camada de húmus e a drenagem é geralmente lenta devido à baixa declividade. A área de restinga com floresta mais fechada apresenta árvores mais altas, podendo atingir de 12 a 15 metros, pouco ramificadas a partir da base. O dossel é mais contínuo, existindo maior quantidade de epífitas (bromeliáceas, orquidáceas e gesneriáceas) e um estrato herbáceo predominantemente constituído de bromélias e orquídeas.

As características fisionômicas da comunidade que compõe a vegetação de restinga estão relacionadas com as condições ambientais da área, notadamente as

edáficas. As condições limitantes do solo refletem-se na vegetação que apresenta escleromorfismo, nanismo e sistema radicular superficial. O componente arbóreo apresenta baixa complexidade estrutural e baixa diversidade específica, com um número pequeno de espécies representado por muitos indivíduos. A família Myrtaceae é que apresenta o maior número de espécies, onde destacam-se *Myrcia bicarinata*, *Myrcia rostrata* e *Eugenia umbelliflora*, entre outras. Outras famílias importantes são: Arecaceae (5 espécies), Melastomataceae (4 espécies), Aquifoliaceae (4 espécies) e Clusiaceae (3 espécies) (Sugyama 1998).

Floresta Pluvial Tropical de Planície Litorânea

A floresta de planície ocorre em continuidade à floresta de restinga periodicamente inundada, sem que seja possível estabelecer limites precisos entre elas. A transição entre a floresta de restinga e a floresta de planície se faz de maneira gradual, caracterizada pelo desaparecimento da vegetação de cobertura do solo, ao mesmo tempo em que a camada de serrapilheira torna-se mais espessa e há uma regressão das características xerofíticas (De Grande & Lopes 1981).

A floresta de planície é composta por dois estratos arbóreos mais ou menos contínuos, dossel fechado, grande quantidade de lianas e epífitas e um estrato arbustivo-herbáceo denso (Barros *et al.* 1991) No estrato arbustivo-herbáceo encontra-se principalmente, *Heliconia velloziana* (Heliconiaceae), *Calathea* spp. (Marantaceae) e *Psychotria nuda* (Rubiaceae). O estrato arbóreo superior pode atingir 20 metros de altura, caracterizado pela presença de *Virola bicuhyba* (Myristicaceae), *Schyzolobium parayba* (Caesalpinaceae) e *Vochysia bifalcata* (Vochysiaceae). O estrato inferior pode atingir 10 metros, onde encontra-se *Mollinedia uleana* (Monimiaceae), *Cabralea*

canjerana (Meliaceae), *Eugenia cuprea*, *Marlieria tomentosa* (Myrtaceae), entre outras (Bernardi *et al.* 2005).

Floresta Pluvial Tropical de Encosta

A floresta pluvial tropical de encosta cobre as encostas e topos mais baixos do maciço montanhoso central e os morros isolados. Essa formação vegetal ocupa a maior extensão, cerca de 74% da Ilha do Cardoso. Estudos realizados por Melo e Mantovani (1994) indicam a ocorrência de 3 estratos arbóreos mais ou menos contínuos: o inferior com 5-10 metros de altura, o médio com 15-21 e o superior com 24-28 metros. Além dos três estratos, ocorrem árvores emergentes com mais de 30 metros e um estrato arbustivo-herbáceo de porte baixo.

A formação de floresta de encosta apresenta variações estruturais muito grandes, por depender de vários fatores como a cota de altitude, os tipos de solo e umidade vinda do oceano. A declividade do terreno não permite que o dossel filtre completamente a luz solar, favorecendo a presença de muitas epífitas (Mantovani *et al.* 1990).

Fauna

A Ilha do Cardoso é um dos maiores sítios de diversidade e riqueza de avifauna da Mata Atlântica, tendo sido registradas 416 espécies de aves ou seja, 53% do total de espécies já registradas para o Estado de São Paulo (Barbosa *et al.* 1988, Willis & Oniki 2003). Entre as aves frugívoras (22,3% das espécies) destacam-se as espécies de grande e médio porte como o macuco *Tinamus solitarius*; o jacuguaçu *Penelope obscura*; a jacutinga *Aburria jacutinga*; o tucano-de-bico-preto *Ramphastos vitellinus*; o tucano-de-

bico-verde *Ramphastos dicolorus*; a araponga *Procnias nudicollis*; o crocroió *Carpornis cuculatus*. Foram registrados cerca de 80 espécies de mamíferos, sendo a maioria roedores e quirópteros, entre os quais, *Lasirus ebenus*, endêmico da Ilha (SMA 1998). Entre as espécies de mamíferos de grande porte estão a lontra (*Lontra longicaudis*), o bugio (*Alouatta guariba*), o veado-mateiro (*Mazama americana*), o queixada (*Tayassu pecari*) e a onça parda (*Puma concolor*). Bernardo & Galetti (2004) avaliaram a abundância de mamíferos cinegéticos presentes na área de estudo, e encontraram que as espécies mais avistadas (encontros/10km) foram: *Alouatta guariba* (1,3); *Dasyprocta leporina* (0,7); *Sciurus ingrami* (0,33); *Nasua nasua* (0,14), *Tayassu pecari* (0,14); *Pecari tajacu* (0,03). A anta (*Tapirus terrestris*) e a onça pintada (*Panthera onca*) foram extintas localmente na década de 60.

Interações frutos-frugívoros

A grande maioria das espécies arbustivas e arbóreas da Ilha do Cardoso depende dos animais para dispersar as suas sementes. Utilizando o levantamento de espécies arbustivas e arbóreas realizado pelo Instituto de Botânica, espécies amostradas por Castro (2007) em um estudo fenológico e espécies consideradas neste estudo, obtivemos informações sobre as síndromes de dispersão para 208 de 265 espécies. A dispersão por animais vertebrados ocorre em 85% destas espécies e a dispersão abiótica em apenas 15%. Esta alta porcentagem de zoocoria é comum em outras áreas tropicais, podendo variar de 70% a 93,5% (ver revisão em Jordano 2000). A porcentagem de espécies zoocóricas na Ilha do Cardoso também é alta em todos os ambientes estudados, 81,45% na restinga arbustiva e arbórea, 84,82% na mata de planície e 86,45% na mata de encosta (espécies que ocorrem em mais de um ambiente foram repetidas). Além dos

animais vertebrados, os invertebrados também são importantes dispersores de sementes. Passos & Oliveira (2003) encontraram que 48 espécies de formigas interagem com 44 espécies de frutos na Floresta de Restinga, sendo assim pode-se sugerir a importância destes animais também nos outros ambientes encontrados na Ilha. Desta forma, a Ilha do Cardoso pode ser considerada um local ideal para estudos de interações frutos-frugívoros, uma vez que a fauna e a flora são relativamente bem conhecidos taxonomicamente, devido a alta diversidade de ambientes e de interações. Essa alta diversidade de interações entre vertebrados e invertebrados frugívoros tem sido apontada como a "arquitetura da biodiversidade" (Bascompte *el al.* 2006). Essas interações, por outro lado, não ocorrem ao acaso e refletem estruturas hierárquicas filogenéticas (Rezende *et al.* 2007)

Florestas tropicais no mundo todo vem sofrendo com a perda de interações entre animais e plantas, especialmente a polinização e dispersão de sementes (Aizen & Feinsinger 1994, Asquith *et al.* 1997, Staggemeier & Galetti 2007), sendo que estes processos são pouco conhecidos (Lopez & Terborgh 2007). A presente tese de doutorado, realizada em um mosaico de ambientes sub-tropical e bem preservado de Floresta Atlântica, avaliou as interações entre vertebrados frugívoros e frutos carnosos sob o ponto de vista ecológico-evolutivo, permitindo uma melhor compreensão sobre os processos que moldam as relações animal-planta.

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Capítulo 1

Phylogenetic effects on components of attractiveness and defense in vertebrate-dispersed fruits

Phylogenetic effects on components of attractiveness and defense in vertebrate-dispersed fruits

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ABSTRACT

Variation in fruit traits has traditionally been identified as adaptations to seed dispersers. This paradigm led to a series of straightforward predictions regarding variation and covariation in fruit characteristics in relation to frugivore quality and preferences. However, the importance of frugivores in shaping fruit traits has been questioned from a number of perspectives. We used comparative methods to investigate the interaction among fruit traits in a broad sample of Atlantic rainforest species. We investigated whether fruit traits related to attraction to seed dispersers and defense against seed predators and pathogens show phylogenetic signal and how these traits interact. We found that morphological and nutritional traits showed phylogenetic signal and in general independent patterns of covariation. On the contrary, fruit color and contrast did not present phylogenetic signal and high-saturated fruits are rich in energy and lipids, and poor in carbohydrates. Thus we suggest that saturated fruits may indicate fruit quality. Therefore, fruit color is an honest signal that might be advantageous to plants by increasing fruit detection and removal. We conclude that in general characteristics such as morphology and chemistry are not the adaptive outcome of natural selection favoring particular combinations of traits over other. Otherwise fruit color may indicate adaptations to frugivores as suggested by earlier naturalists.

Keywords fruit color, phylogenetic signal, plant-animal interaction, secondary compounds

Introduction

Fruit characteristics, such as morphology and pulp nutrient composition, have traditionally been identified and interpreted as reflecting broad adaptation to seed dispersers (Ridley 1930, van der Pijl 1969, Howe 1986, Fleming *et al.* 1993). But the importance of frugivores in shaping fruit traits has now been questioned from a number of perspectives. Genetic constrains (Howe 1984), phylogeny and history (Herrera 1986, 1992, Janson 1992) have been identified as the main obstacles for the evolution of tight coevolved relationships between fruits and their seed dispersers. Coevolution between frugivores and fruiting plants also appears to be constrained by inconsistent selection pressures over space and time (Herrera 1998).

On the basis of historical and phylogenetic effects, the current belief is that seed dispersers are neither strongly selecting any fruit traits nor promoting evolutionary radiation (Herrera 1985). In one of the most extensive studies on fruit traits, Jordano (1995) found a lack of evolutionary correlation between seed dispersal type and most fleshy fruit traits; in fact only seed diameter showed a trend for covariation with type of seed disperser when taken into account plant phylogeny. But the lack of tight plant-disperser coevolution may be explained by other factors as well. Plant-disperser interactions do not take place in a vacuum; many other interactions may simultaneously shape the evolution of both partner's traits (Herrera 1986). Most previous studies of fruit-seed dispersers interactions have been limited by focusing solely upon vertebrate seed dispersers. Many potentially detrimental organisms, such as invertebrate predators, fungus and bacteria, may compete with legitimate seed dispersers for fruit resources (Janzen 1977, Herrera 1982). Their effects on the evolution of fruit traits will be different than, and even opposite to, the effect of seed dispersers. Viewed in this

context, plants are faced with an evolutionary dilemma: how to make fruits simultaneously attractive to mutualistic partners (seed dispersers) but unattractive to all antagonists (seed predators and pathogens). Secondary compounds have long been viewed as mediators of this conflict (Janzen 1977, Herrera 1982, Cipollini & Stiles 1992, Levey *et al.* 2006).

Plant traits directed to select the most appropriate disperser among the set of potential vertebrates have been often discussed in the literature (Snow 1971, McKey 1975, Howe & Estabrook 1977, MacCarty *et al.* 2002, Izhaki 2002). Nevertheless, the possible adaptations evolved by plants to defend their ripe fruits from pathogens and predators remain poorly explored. Some studies have evaluated how the contents of nutrients and secondary compounds affect fruit removal by frugivores (Tang *et al.* 2005, Schaefer et al. 2003, Cazetta *et al.* in press). However, the relationship among these traits or other traits related to fruit attractiveness, such as morphology and color, have been neglected in most studies of fruit characteristics (but see Schaefer & Schmidt 2004).

Interspecific variation among ecologically important plant traits may reflect the adaptive outcome of natural selection favoring particular combinations of traits over others. Thus, interaction between fruit traits might suggest new insights about natural selection on these characteristics. In this study we evaluated the interactions among fruit traits in a broad sample of Atlantic rainforest species. We are mainly interested in variation on traits related to attraction to frugivores and defense against pathogens. However, phylogenetic effects can act on phenotypic traits by imposing a rigid pattern of covariation among them. Phylogenetic comparative methods are the main tool to examine patterns and to infer process of evolutionary change (Garland *et al.* 2005). A

large set of comparative methods (Felsenstein 1985, Pagel and Harvey 1988, 1989, Martins & Garland 1991, Garland *et al.* 1992, 1993) can be used for assessing the evolutionary association among quantitative characters. In the present study we used comparative methods to assess the evolutionary association among fruit traits. Our main goals were: (1) To evaluate whether different fruit variables, such as fruit morphology, chemistry, color, and defense, show significant phylogenetic signal and quantify the amount of signal for each variable; and (2) To determine how these phenotypic traits are correlated after accounting for phylogeny. In this context, we tested to what extent components of attractiveness and defense are inherited as sets of traits, and how attractiveness and defense interact. We predicted that: (a) smaller fruits have more contrasting color than large fruits, and (b) low contrasting fruits have higher contents of secondary compounds or higher contents of nutrients than higher contrasting fruits.

Material and Methods

Study site

Fruits were collected at Cardoso Island (25°03' S and 48°53' W), an inshore island, located in the south of São Paulo state, Brazil. Cardoso Island is a 15,100 ha protected island represented by several types of Atlantic rainforest, including lowland and highland tropical rainforest, mangroves, dune vegetation and restinga forest (Barros *et al*. 1991).

This study was carried out in restinga (sandy forest), lowland, and highland tropical rainforest. Restinga forest is an ecosystem of Atlantic rainforest biome that belongs to the group of pioneer formations with marine influence and that is distributed over a quaternary coastal plain (Ab'Saber 1955). It constitutes a heterogeneous ecosystem (Lima & Capobianco 1997) encompassing a mosaic of vegetation types that

varies from open areas to forest formations and from low to medium canopy height (3-15 m). In sequence, the forest formation presents a higher density of trees and is named lowland tropical rainforest. It is characterized by an understory level and a relatively dense and continuous canopy that can reach 20 m with large amounts of epiphytes and vines (Barros *et al.* 1991). The highland tropical rainforest covers the slopes of central mountains and occupies almost 70% of the whole island. This forest formation is characterized by clay soil and presence of three continuous arboreal strata: lower (5-10 m), medium (15-21 m), and high (24 -28 m).

The climate at Ilha do Cardoso is generally warm and wet throughout the year without a marked dry season (Funari *et al*. 1987). Mean annual rainfall can reach 3000 mm and mean annual temperature is 23.8^{o}C (Castro *et al.* 2007).

Study Species

We collected quantitative information on fleshy fruit traits of 105 species from 44 different families (Appendix I). The sample was dominated by trees (56.19%), followed by shrubs (27.62 %), vines (7.62%), herbs (4.76%), hemiparasites (1.90%), and bromeliads (1.90%). Although we evaluated fruit morphology of all the species (Appendix II), data on fruit chemistry were available for 78 species (Appendix III) and on fruit color (i.e. fruit reflectance and chromatic and achromatic contrasts for birds and primates) for 58 species (Appendix IV). The species represent approximately 50% of the known vertebrate-dispersed plants on Cardoso Island. Taxonomically, the sample was dominated by Myrtaceae (18 species), Rubiaceae (13 species) and Arecaceae (8 species). Fruit species are mainly dispersed by birds (72.38%) followed by mixed

agents (14.29%) and mammals (13.33%), a common pattern found in the Atlantic rainforest vertebrate-dispersed fruits (Almeida-Neto *et al.* in press).

Methods

Morphological traits

We collected 30 fruits of at least three different individuals to assess fruit morphology. For each fruit we recorded: 1. length and diameter of fruits and seeds (mm); 2. fruit dry and fresh mass (g); 3. seed mass (g); 4. number of seeds per fruit. From these measurements we calculated two ratios: 1. relative yield of a fresh fruit (dry mass of the pulp/fresh mass of whole fruit); 2. pulp to seed ratio (dry mass of pulp/mass of the seeds).

Chemical analyses

For the chemical analyses, fruit pulp or aril from at least three individuals of each species was frozen until the moment of analysis. Proteins were determined according to the Kjeldahl method, in which total protein is calculated by multiplying total nitrogen by 6.25 (Jeffery *et al.* 1989). Lipid content was determined according to the macro-gravimetric method (Bligh & Dyer 1959) and the contents of glucose, fructose, and sucrose by gas chromatography- mass spectrometer (modified from Pooter & Villar 1997). We used the mean gross energy equivalents of protein (17.2 KJ/g), lipid (38.9 KJ/g), and sugar (17.2 KJ/g, Karlson 1972) to determine the energetic value of dried fruit pulp for each species.

We also determined the contents of phenols and condensed tannins in fruits. We focused on phenols because they commonly occur in fleshy fruits and they are the most widespread secondary compounds in ripe fruits (van Buren 1970, Herrera 1982,

Cipollini & Stiles 1992, Foley et al. 1995). They possess antimicrobial and antifungal activities (Davidson & Juneja 1990, Scalbert 1991), they affect fruit persistence, and may reduce seed dispersal and overall attractiveness (Cazetta *et al.* in press). We extracted these compounds by the Price and Butler (1977) method in butanol and methanol extracts (see Schaefer *et al.* 2003). The contents of these compounds were analyzed with photometric measurements. For each fruit sample, we ran three replicates and used the mean of the three values for statistical analyses.

Color measurements and contrast calculation

We measured the reflectance spectra of 15 fruits, 10 leaves and 10 accessory structures when present. We performed all measurements with an Ocean optics USB2000 spectrometer and a Top Sensor System Deuterium-Halogen DH-2000 (both Ocean Optics, Duiven, Netherlands) as a standardized light source. Reflectance was measured as the proportion of a standard white reference tile (Top Sensor Systems WS-2).

We calculated three variables that characterize the reflectance spectra: the achromatic intensity (total brightness), color saturation (hereafter chroma) and hue (Endler 1990). In addition, we calculated chromatic and achromatic contrasts between the mean reflectance of fruits and leaves or secondary structure according to eye models of avian and primate vision. Both models assume that receptor noise limits discrimination (Vorobyev & Osorio 1998, Vorobyev *et al.* 2001). Because passerine birds are the most important frugivorous birds on our study site and on neotropical forests in general (Loiselle & Blake 1991), we used an eye model based on the spectral sensitivities of a passerine bird, the blue tit (*Cyanistes caeruleus*) with a UVS cone (Hart *et al.* 2000). We used the spectral sensitive of humans because these correspond to

those of *Alouatta* sp., which is the only primate species in our study site. Based on analytical approximation of cone visual pigments and oil droplet spectra, we calculated the quantum catch of each class of single cones (LWS, MWS, SWS, UVS) for birds, and based only on analytical approximation of cone visual pigments, we calculated the quantum catches (LWS, MWS, SWS) for primates, denoted by the subscript *i*, as the integrated product of the receptor sensitivity spectrum (R*i*), reflectance spectrum (S), and illumination spectrum (I):

(1) $Qi = Ri(\lambda)S(\lambda)I(\lambda)d\lambda$

The quantum catches are used to find relative contrasts against fruits and background as the log of the quotient of quantum catches from both spectra. The result of this calculation is the contrast Δf for each receptor type *i*:

(2) $\Delta fi = \ln(Qi \text{ fruit}) - \ln(Qi \text{ background}) = \ln(Qi \text{ fruit}/Qi \text{ background})$

To quantify discrimination using all receptor types in a given visual system, each receptor class is first assigned a noise value ω based on its individual Weber fraction (v) and on the receptor proportion (n) (Vorobyev *et al.* 2001):

(3) $\omega i = vi/ni$

Then we calculated discrimination values for trichromatic (4i) and tetrachromatic (4ii) visual system, respectively. The subscript number of each variable in equation 4 is the value given for a particular receptor class (1 to 3 for primates and 1 to 4 for birds):

(4i) $\Delta S^2 = [(\omega 1^2 (\Delta f3-\Delta f2)^2 + (\omega 2^2 (\Delta f3-\Delta f1)^2 + (\omega 3^2 (\Delta f1-\Delta f2)^2] / [(\omega 1\omega 2)^2+(\omega 1\omega 3)^2+(\omega 2\omega 3)^2]$

(4ii) $\Delta S^2 = [(\omega1\omega2)^2 (\Delta f4-\Delta f3)^2 + (\omega1\omega3)^2 (\Delta f4-\Delta f2)^2 + (\omega1\omega4)^2 (\Delta f3-\Delta f2)^2 + (\omega2\omega3)^2 (\Delta f4-\Delta f1)^2 + (\omega2\omega4)^2(\Delta f3-\Delta f1)^2 + (\omega3\omega4)^2 (\Delta f2-\Delta f1)^2] / [(\omega1\omega2\omega3)^2+(\omega1\omega2\omega4)^2+(\omega1\omega3\omega4)^2+(\omega2\omega3\omega4)^2]$

Results of equation 4 provide the chromatic distance (ΔS) separating the perceptual values of two spectra in receptor space. The units for ΔS are jnds (just noticeable differences), where 1 jnd is at the threshold of discrimination, values less than 1 jnd indicate that two colors are indistinguishable and values above 1 can be discriminated (Osorio & Vorobyev 1996).

The achromatic (brightness contrast) analysis is similar to the chromatic one, where comparisons are based on brightness differences alone:

$\Delta S = |\Delta fi/\omega|$

Data analysis

For all statistical analyses, all variables were log transformed, except for water and sugar that were Arcsin(SqRt) and square root transformed, respectively. We performed three separate principal component analyses (PCA) to evaluate major independent trends in the variation of morphological, chemical and color fruit traits.

We reconstructed the phylogeny of all plant species that we evaluated using Phylomatic software (Webb & Donoghue 2005). The phylogenies used for this study were based on the Phylomatic conservative tree. Because estimates of divergence times were not available, we tested three different types of arbitrary branch lengths: all = 1 (constant), Granfen's (1989) and the variant Nee's (Purvis 1995), and Pagel's (1992). Statistical adequacy of the different branch lengths were tested by plotting the absolute values of standardized phylogenetic independent contrasts against their standard

deviations. Branch lengths are statistically adequate when there is no correlation between these values (Garland *et al.* 1992).

We tested for the presence of phylogenetic signal in our continuous-valued traits and also in all principal components, using the randomization procedure in the Matlab program PHYSIG.M of Blomberg *et al.* (2003). This method consists in shuffling tip values across the real phylogeny and obtaining the distribution of variance in contrasts under this null model. If the variance of the real data set is lower than 5% of the values obtained from the randomizations (1000 in our study), we rejected the null hypothesis of no signal with $P < 0.05$. We also reported the *k*-statistic to describe the amount of phylogenetic signal in the continuous-valued traits and in the principal components (Blomberg *et al.* 2003). A value of 1 indicates exactly the amount expected under Brownian motion evolution along the specific topology and branch lengths of the tree. Values < 1 indicate less resemblance of relatives than expected, and values > 1 indicate stronger than expected phylogenetic clumping of trait values.

We used the Matlab program REGRESSIONV2.M (Garland & Ives pers. comm.) to perform linear statistical models via both ordinary least squares (OLS) (i.e. non-phylogenetic) and phylogenetic generalized least square regressions (PGLS) (Garland & Ives 2000, Garland *et al.* 2005). Ordinary least square regression assumes that the unexplained residual variation is independent among species, whereas PGLS assumes that residual variation among species is correlated, with the correlation given by the degree of phylogenetic relatedness between species. We also performed 3 transformed models: Granfen's ρ, Pagel's λ, Ornstein-Uhlenbeck - OU (as derived in Blomberg *et al.* 2003). The program estimates the optimal transformation parameter (ρ, λ and *d*), using restricted maximum likelihood (REML). A parameter value of one

indicates that the statistical model with the original branch lengths (i.e. the PGLS model) best fits the data; a value of zero indicates that a star phylogeny (i.e. the OLS model) best fits the data; and a parameter between 0 and 1, which is most typically found, indicates that branch lengths intermediate between the starter and a star phylogeny best fits the data.

Employing these multiple regressions, we tested for correlations in the principal components for chemistry, morphology and color obtained from PCA analyses. Initial models included all principal components, and we subsequently removed those variables that were not statistically significant (this is analogous to a backward regression). For results of all models carried out see appendix A. We compared the different models using the Akaike Information Criterion (AIC), using the smaller-is-better formulation [AIC = -2*ln maximum likelihood) + (2* # of parameters)]. When comparing a series of models, the one with the lowest AIC is considered to be the best.

Results

Fruit traits

The PCA on morphological traits revealed that the first three components accounted for 92.36% of total variance. Morphological traits related to fruit and seed size (diameter and length) and fruit and seed mass are highly correlated (Appendix B). Variation in these traits represented the first principal component (Morph_PC1) explaining 55.44% of the overall variation. The second principal component (Morph_PC2) is associated with seed number and pulp/seed. Fruits with high relative yield show high values on the third principal component (Morph_PC3) (Table 1).

The PCA for nutritional traits identified three components that accounted for 80.74% of total variance. Energy was positively correlated with lipid and negatively correlated with carbohydrate and water (Appendix C). Variation in these traits represented the first principal component (Chem_PC1), explaining 39.59% of the variation. Fruits with high contents of phenols and condensed tannins scored high on the second principal component (Chem_PC2), explaining 25.85% of the overall variation. Fruits with high protein contents scored high on the third principal component (Chem_PC3) (Table 1).

The PCA on color traits identified two principal components that account for 64.18% of total variance. Chromatic and achromatic contrasts for primates and birds are highly correlated (Appendix D). These traits score high on the first principal component (Col_PC1) and low values of hue and brightness also score high on this component. Fruits with low color saturation (chroma) show high values on the second principal component (Col_PC2) (Table 1).

Phylogenetic signal

Our phylogeny presented little hierarchical structure (Figure 1). The branch length that showed better adequacy for all traits was Nee's arbitrary branch lengths transformed with Grafen (1989)'s rho of 0.5 (Table 2). Randomization tests suggest a significant phylogenetic signal in morphological traits, except for pulp fresh mass. We also found phylogenetic signal in chemical traits, such as lipids, proteins and energy contents, but not in carbohydrates contents. There was no phylogenetic signal in the defense traits (phenols and tannins contents) nor in any of the color traits evaluated. All morphological and chemical principal components showed phylogenetic signal, except

Chem_PC3. There was no phylogenetic signal in color principal components (Table 2). The *K*-statistics revealed that the amount of signal is lower than expected by Brownian motion for all fruit traits evaluated (Table 2).

Table 1 Principal component analyses of morphological, nutritional and color fruit traits from Cardoso Island, Atlantic rainforest, Brazil (factor loading >0.5 are shown).

Variable		***Factors***	
Morphological Traits	**Morph_PC1**	**Morph_PC2**	**Morph_PC3**
Fruit fresh mass	0.97		
Fruit length	0.94		
Fruit diameter	0.92		
Pulp dry mass	0.92		
Pulp fresh mass	0.91		
Seed mass	0.89		
Seed diameter	0.71	-0.64	
Seed length	0.69	-0.65	
Seed number		0.87	
Pulp/seed		0.83	
RY			0.91
Eingenvalues	6.1	2.82	1.23
% variance explained	55.45	25.68	11.23
% cumulative variance	55.45	81.13	92.36
Nutrient contents	**Chem_PC1**	**Chem_PC2**	**Chem_PC3**
Energy	0.95		
Lipids	0.94		
Sugar	-0.65		
Water	-0.62		0.54
Tannins		0.91	
Phenols		0.85	
Proteins			0.74
Eingenvalues	2.77	1.81	1.07
% variance explained	39.56	25.85	15.32
% cumulative variance	39.56	65.41	80.74
Color and contrasts	**Col_PC1**	**Col_PC2**	
Achromatic primate	0.86		
Chromatic primate	0.82		
Achromatic bird	0.75		
Chromatic bird	0.8		
Brightness	-0.62		
Hue	-0.55		
Chroma		-0.72	
Eingenvalues	3.45	1.04	
% variance explained	49.25	14.93	
% cumulative variance	49.25	64.18	

Relationship among fruit traits

The non-phylogenetic OLS models always provided better fits than all phylogenetic models when evaluating the relationship between morphological principal components versus chemical and color principal components. Moprh_PC1 and Morph_PC2 were not correlated with any of the chemical and color components. Morph_PC3 was correlated with Chem_PC2 and Chem_PC3 (Table 3; Figure 2).

Chemical principal components were not correlated with morphological and color components, except for the same relationships described above (Chem_PC2 and Chem_PC3 with Morph_PC3). The PGLS and Ornstein-Uhlenbeck – OU model fits better the relationship between Chem_PC1 and Chem_PC2 (respectively) versus morphological and color components. By contrast, the non-phylogenetic model fits better the relationship between Chem_PC3 versus morphology and color.

The non-phylogenetic model also provided a better fit in the relationship between Col_PC1 versus morphological and chemical components. This component was not correlated with any of the variables evaluated. Col_PC2, related with fruit chroma, was correlated with Chem_PC1, so that fruits with high color saturation values are rich in lipids and energy, and poor in water and carbohydrates (Table 3; Figure 3).

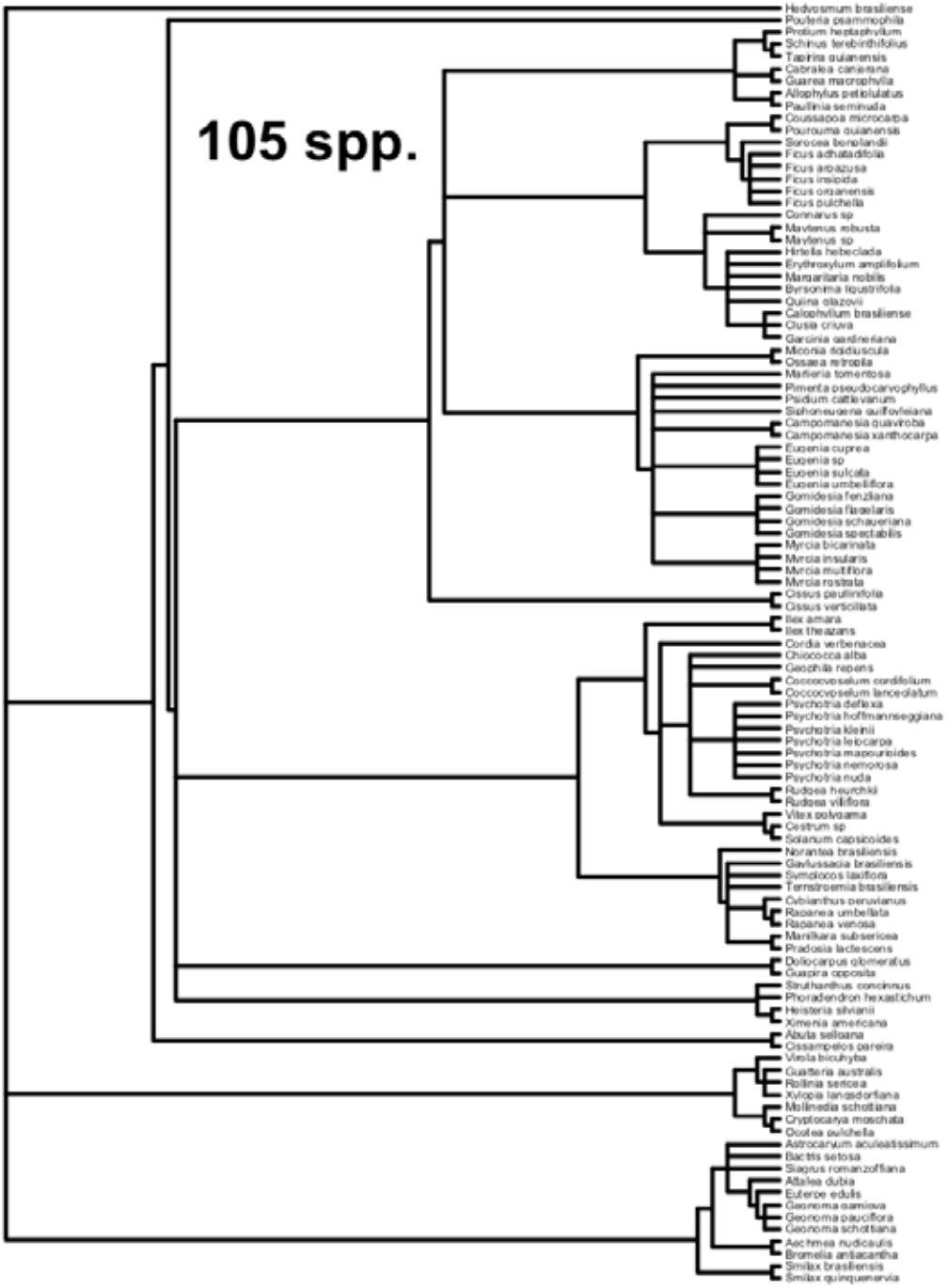

Figure 1 Phylogeny of the vertebrate-dispersed species at Cardoso Island, Atlantic rainforest, Brazil.

Table 2 Phylogenetic signal and *K*-statistic for fruit phenotypic traits on Cardoso Island. All arbitrary branch lengths selected were Nee with 0.5 rho transformation. Significant phylogenetic signal are typed in boldface.

Type	Phenotypic trait	N	Diagnostic r	*K*	*P*
Morphology	Fruit diameter	105	0.1134	0.7511	**0.044**
	Fruit length	105	0.0653	0.7798	**0.017**
	Fruit mass	105	0.2361	0.7702	**0.027**
	Pulp fresh mass	105	0.2877	0.7358	0.076
	Pulp dry mass	105	1.8412	0.8643	**< 0.001**
	Seed Diameter	105	0.365	0.8963	**< 0.001**
	Seed length	105	0.4251	0.8732	**0.001**
	Seed number	105	0.5063	0.9214	**<0.001**
	Seed mass	105	0.3391	0.8222	**0.004**
	Pulp/Seed	105	0.0633	0.8275	**0.005**
	RY	105	0.0017	0.8065	**0.003**
Nutrients	Water	78	0.1007	0.8444	**0.007**
	Lipids	78	1.5539	0.9096	**0.001**
	Proteins	78	0.8704	0.8391	**0.023**
	Sugar (Glu+ Fru + Su)	78	0.0589	0.7831	0.077
	Energy	78	1.8713	0.8915	**0.005**
Color	Chromatic Bird	58	0.0219	0.7603	0.181
	Achromatic Bird	58	1.2363	0.7032	0.505
	Chromatic Primate	58	0.9831	0.69	0.637
	Achromatic Primate	58	0.4675	0.683	0.682
	Brightness	58	0.5057	0.7998	0.082
	Hue	58	0.1648	0.7187	0.419
	Chroma	58	0.0048	0.7533	0.27
Defense	Phenols	78	0.2744	0.7668	0.074
	Tannins	78	0.0044	0.7778	0.078
Principal components	Morph_PC1	105	0.1008	0.7768	**0.014**
	Morph_PC2	105	0.99	1.0065	**<0.001**
	Morph_PC3	105	0.9515	0.7618	**0.026**
	Chem_PC1	78	1.2471	0.9303	**<0.001**
	Chem_PC2	78	0.0292	0.7964	**0.036**
	Chem_PC3	78	0.3871	0.7549	0.176
	Col_PC1	58	0.4098	0.7429	0.27
	Col_PC2	58	0.0522	0.6795	0.692

Table 3 Results from multiple regressions using Regressionv2.m. Two models are shown: ordinary least square (OLS) and the best phylogenetic model selected (PGLS, Granfen's ρ, Pagel's λ or Ornstein-Uhlenbeck – OU) by criterion of lowest AIC. Significant relationships are typed in boldface.

Dependent	Independent	BL transform	AIC	
Morph_PC1	Chem_PC3	OLS	134.14	$F_{1,44}$ = 1.63039, P = 0.208
	Col_PC1			$F_{1,44}$ = 1.80295, P = 0.186
Morph_PC1	Chem_PC3	OU (d= 0.270)	135.55	$F_{1,44}$ = 1.48094, P = 0.230
	Col_PC1			$F_{1,44}$ = 1.57690, P = 0.216
Morph_PC2	Chem_PC1	OLS	138.49	$F_{1,44}$ = 2.31938, P = 0.135
	Col_PC1			$F_{1,44}$ = 3.50084, P = 0.068
Morph_PC2	Chem_PC1	OU (d= 0.381)	140.16	$F_{1,44}$ = 1.47302, P = 0.231
	Col_PC1			$F_{1,44}$ = 3.10726, P = 0.085
Morph_PC3	Chem_PC1	OLS	111.07	$F_{1,41}$ = 3.78244, P = 0.059
	Pchem2			**$F_{1,41}$ = 9.69415, P = 0.003**
	Chem_PC3			**$F_{1,41}$ = 10.8060, P = 0.002**
	Col_PC1			$F_{1,41}$ = 0.51940, P = 0.475
	Col_PC2			$F_{1,41}$ = 0.36149, P = 0.551
Morph_PC3	Chem_PC1	Pagel	113.07	$F_{1,41}$ = 3.78244, P = 0.059
	Pchem2	(=2.602)		**$F_{1,41}$ = 9.69415, P = 0.003**
	Chem_PC3			$F_{1,41}$ = 0.10806, P = 0.744
	Col_PC1			$F_{1,41}$ = 0.51940, P = 0.475
	Col_PC2			$F_{1,41}$ = 0.36149, P = 0.551
Chem_PC1	Morph_PC3	OLS	138.14	$F_{1,44}$ = 2.55714, P = 0.117
	Col_PC2			$F_{1,44}$ = 2.68515, P = 0.108
Chem_PC1	Morph_PC3	PGLS	136.79	$F_{1,44}$ = 1.95263, P = 0.169
	Col_PC2			$F_{1,44}$ = 3.69560, P = 0.061
Chem_PC2	Morph_PC3	OLS	126.06	**$F_{1,43}$ = 6.34153, P = 0.016**
	Col_PC1			$F_{1,43}$ = 0.57270, P = 0.453
	Col_PC2			$F_{1,43}$ = 0.54726, P = 0.463
Chem_PC2	Morph_PC3	OU (*d*= 0.412)	125.95	**$F_{1,43}$ = 5.82240, P = 0.020**
	Col_PC1			$F_{1,43}$ = 0.69217, P = 0.410
	Col_PC2			$F_{1,43}$ = 0.20234, P = 0.655
Chem_PC3	Morph_PC1	OLS	135.19	$F_{1,43}$ = 0.51803, P = 0.476
	Morph_PC3			**$F_{1,43}$ = 7.45999, P = 0.009**
	Col_PC1			$F_{1,43}$ = 0.82509, P = 0.369

Dependent	Independent	BL transform	AIC	
Chem_PC3	Morph_PC1	Pagel	137.19	$F_{1,43}$ = 0.51803, P = 0.476
	Morph_PC3	(= 2.602)		**$F_{1.43}$ = 7.45999, P = 0.009**
	Col_PC1			$F_{1,43}$ = 0.82509, P = 0.369
Col_PC1	Morph_PC1	OLS	129.31	$F_{1,43}$ = 1.58128, P = 0.215
	Morph_PC2			$F_{1,43}$ = 3.08175, P = 0.086
	Chem_PC3			$F_{1,43}$ = 1.10668, P = 0.299
Col_PC1	Morph_PC1	OU (*d*= 0.181)	131.15	$F_{1,43}$ = 1.34568, P = 0.252
	Morph_PC2			$F_{1,43}$ = 2.83026, P = 0.099
	Chem_PC3			$F_{1,43}$ = 1.08234, P = 0.304
Col_PC2	Chem_PC1	OLS	130.9	$F_{1,45}$ = 3.20197, P = 0.080
Col_PC2	Chem_PC1	Grafen (ρ=0.010)	130.44	**$F_{1,45}$ = 6.51240, P = 0.014**

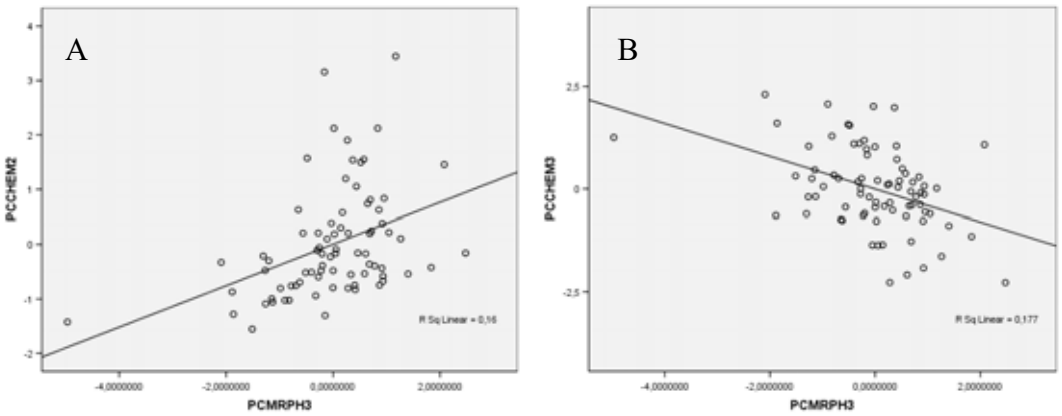

Figure 2) Relationships between (A) MORPH_PC3 and CHEM_PC2, and (B) MORPH_PC3 and PCHEM3. Correlations statistics are given in table 3.

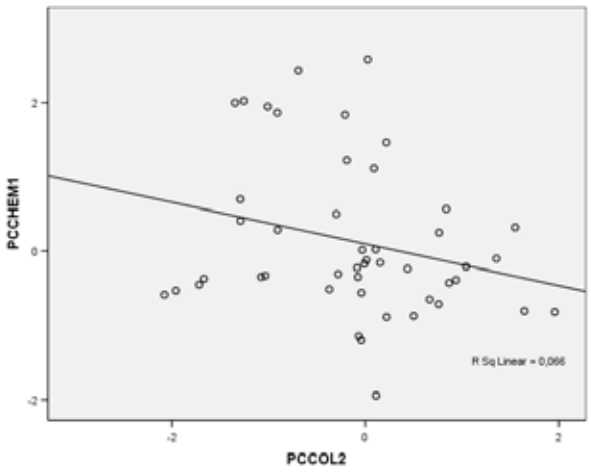

Figure 3) Relationship between COL_PC2 and CHEM_PC1. Statistics are given in table 3.

Discussion

Morphological, nutritional, and color fruit patterns

In three separate PCA, three morphological and nutritional components and two color components explained 92.36%, 80.74%, and 64.18% of the total variance in fruit traits, respectively. Fruit size and mass defined the major principal component (Herrera 1987, Fuentes 1994) but also seed size and mass. The second component was correlated with pulp/seed and seed number as also found by Schaefer *et al.* (2003). Finally, the third component was correlated with the relative yield of the fruit. The contents of lipids and energy were negative correlated with carbohydrates and were identified as the major principal nutritional component, as also found in previous studies (Herrera 1987, Fuentes 1994, Jordano 1995). The contents of phenols and condensed tannins accounted for the second nutritional component that explained 25.85% of total variance. Schaefer *et al.* (2003) found a very similar result with phenols and tannins explaining 26% of the variance of the second nutritional component. We suggest that the variance in secondary

compounds represent a major line of evolutionary diversification in fleshy fruits. The third nutritional component was correlated with protein contents. Chromatic and achromatic contrasts as viewed by birds and primates and fruit brightness were the major principal color component and fruit saturation accounted for the variation in the second color component. Comparable quantitative studies on fruit color are still lacking, but analyzing fruit spectra reflectance, Schaefer & Schmidt (2004) also found that fruit brightness was responsible for the major variation in the first principal component.

Phylogenetic signal

We found phylogenetic signal in most morphological and nutritional fruit traits evaluated, except for pulp fresh mass, carbohydrates, phenols and tannins, and all color traits. However, phenols and tannins are marginally significant and when we tested the component responsible for these two traits the signal was detected. The pattern found, was expected for fruit morphology because usually morphological traits tend to be more conserved along the phylogeny, and errors tend to be lower in morphological measurements. However, as far as we know, this is the first study to evaluate phylogenetic signal for fruit chemical characteristics. The probability of detecting signal depends on the number of species and on the *K* values (Blomberg *et al.* 2003). In a revision study, Blomberg *et al.* (2003) found that 90% of the evaluated traits with sample sizes greater than 20 showed statistically significant phylogenetic signal. Therefore, we are confident that sample size was not a limiting factor in detecting signal for our traits. Despite the fact that our tree was little hierarchical, which implies that most of the evolutionary history is independent between species, we were able to detect signal when it was present.

The presence of significant signal on fruit traits might reflect the existence of phenotypic constrains or historic effects, but it does not require a failure to evolve (Wake 1991). Neither the descriptive statistic (*K*) nor the test for phylogenetic signal attempt to take into account effects of adaptation (Blomberg & Garland 2002). For the same reason, we cannot affirm that lack of significant signal means adaptation to frugivores. To infer adaptation is necessary to correlate character variation and continuous or categorical descriptors that can indicate variation in selective regime. However, the lack of signal for all fruit color traits and also all principal components related to fruit color support previous studies that considered fruit color as adaptations to frugivores. Sustaining this adaptive framework, broad interspecific comparisons yielded that phylogenetic constraints on fruit colors are weak compared to most other fruit traits (Voigt *et al.* 2004).

Patterns of fruit trait relationship

This is the first study to evaluate the relationship between fruit characteristics related to attractiveness and defense in a broad plant community. We found weak character association when we take or not the phylogenetic relationship among species into account. In our study, we found that many times the OLS model (non-phylogenetic model) fits better than all phylogenetic models evaluated. This might be due to the little hierarchical phylogeny but mainly because the correlations include variables without phylogenetic signal (e.g. fruit color).

To a large extent, morphological traits show independent patterns of covariation relative to nutritional traits. Jordano (1995) also found a marked decoupling of fruit morphology characteristics and nutrient content characteristics. In fact, our results

showed that the only pattern of covariation among morphology and nutrients was that fruits with high relative yield tend to contain a larger amount of nutrients and secondary compounds and a lower amount of protein and water. The relative yield indicates how much dry material is gained per unit mass of whole fruit ingested and processed. It seems intuitively obvious that increasing the proportion of dry material would enhance the gain of nutrients. Nonetheless, the negative relationship between relative yield and Chem_PC3 is explicable because there is a fraction of water variation in this component that clearly influences the relationship.

Our results did not support the prediction that highly contrasting fruits are less costly in nutrients, probably because it was too simplistic. If high contrasting fruits had lower nutritional gains, consumers would quickly learn to avoid non-rewarding fruits because they are able to associate the context to the nutritional gain (Hurly & Healy 1996). We also did not find that small fruits are more contrasting as suggested by Willson & Thompson (1982), and Herrera (1987). These authors found that bicolored fruit displays occur more often among species with small fruits but in spite of our findings that bicolored fruits are more contrasting (data not shown) they are not more often in small fruits. Plants also did not signal secondary compounds because, given that dispersers avoid phenols and tannins in fruits (Schaefer *et al.* 2003, Cazetta *et al.* in press) secondary compounds-related signal would not increase plant's fitness. The main relationship found in this study was between color saturation and fruit nutrients. Fruits with high-saturated colors are rich in energy and lipids and poor in carbohydrates. Thus, we suggest that saturated colors may indicate fruit quality. Besides, a previous study in the same area showed that birds preferentially consume fruits rich in lipids and energy (Cazetta *et al.* in press). Although studies evaluating fruit color as signal are still scarce,

at least in some communities, where plants compete for dispersal services by animals, plants use color to signal the presence of macro-nutritional rewards (e.g. proteins, carbohydrates; Schaefer & Schmidt 2004). Recently, Schaefer *et al.* (in press) also found that fruit color is an honest signal of antioxidants. We suggest that plants honestly signal nutritional rewards to attract seed dispersers, which in turn can increase plant's reproductive success by increasing fruit detection and removal.

Conclusions

We have demonstrated that in general morphological and nutritional traits showed significant phylogenetic signal and they are weak associated. Accordingly, a previous study also showed that morphology and chemistry are decoupled traits (Jordano 1995). Therefore, natural selection is not favoring particular combinations of morphological and chemical traits over others. Our study also suggests that fruit traits related to attractiveness and defense were not correlated. On the contrary, fruit color traits did not present phylogenetic signal and consistently with other studies might indicate fruit adaptation to frugivores. Moreover, color saturation is an honest signal of fruit contents that might be advantageous to plants by increasing fruit detection and removal.

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Appendix A Multiple regressions for all models analyzed: ordinary least square (OLS), phylogentic generalized least square (PGLS), Granfen's ρ, Pagel's λ and Ornstein-Uhlenbeck (OU).

Dependent	Independent	BL transform	AIC	
Morph_PC1	Chem_PC1	OLS	137.01	$F_{1,41}$ = 1.12732, P = 0.295
	Pchem2			$F_{1,41}$ = 1.14806, P = 0.290
	Chem_PC3			$F_{1,41}$ = 1.47698, P = 0.231
	Col_PC1			$F_{1,41}$ = 1.85535, P = 0.181
	Col_PC2			$F_{1,41}$ = 1.05852, P = 0.309
Morph_PC1	Chem_PC1	PGLS	141.52	$F_{1,41}$ = 1.50590, P = 0.227
	Pchem2			$F_{1,41}$ = 0.37443, P = 0.544
	Chem_PC3			$F_{1,41}$ = 0.91063, P = 0.345
	Col_PC1			$F_{1,41}$ = 1.18468, P = 0.283
	Col_PC2			$F_{1,41}$ = 0.44240, P = 0.509
Morph_PC1	Chem_PC1	OU (*d*=0.207)	138.75	$F_{1,41}$ = 1.32465, P = 0.256
	Pchem2			$F_{1,41}$ = 0.83840, P = 0.365
	Chem_PC3			$F_{1,41}$ = 1.34887, P = 0.252
	Col_PC1			$F_{1,41}$ = 1.67911, P = 0.202
	Col_PC2			$F_{1,41}$ = 0.85217, P = 0.361
Morph_PC1	Chem_PC1	G (r=0.029)	138.2	$F_{1,41}$ = 1.87693, P = 0.178
	Pchem2			$F_{1,41}$ = 1.29222, P = 0.262
	Chem_PC3			$F_{1,41}$ = 0.93145, P = 0.340
	Col_PC1			$F_{1,41}$ = 1.97217, P = 0.168
	Col_PC2			$F_{1,41}$ = 0.94646, P = 0.336
Morph_PC1	Chem_PC1		139.01	$F_{1,41}$ = 1.19789, P = 0.280
	Pchem2			$F_{1,41}$ = 0.97599, P = 0.329
	Chem_PC3			$F_{1,41}$ = 1.41718, P = 0.241
	Col_PC1			$F_{1,41}$ = 1.65108, P = 0,206
	Col_PC2			$F_{1,41}$ = 0.93028, P = 0,340
Morph_PC1	Chem_PC1	OLS	135.58	$F_{1,43}$ = 0.51776, P = 0.476
	Chem_PC3			$F_{1,43}$ = 1.51447, P = 0.225
	Col_PC1			$F_{1,43}$ = 1.82792, P = 0.183
Morph_PC1	Chem_PC1	PGLS	138.39	$F_{1,43}$ = 1.02912, P = 0.316
	Chem_PC3			$F_{1,43}$ = 0.87715, P = 0.354
	Col_PC1			$F_{1,43}$ = 1.32759, P = 0.256
Morph_PC1	Chem_PC1	OU (*d*= 0.307)	136.76	$F_{1,43}$ = 0.79046, P = 0.379
	Chem_PC3			$F_{1,43}$ = 1.29099, P = 0.262
	Col_PC1			$F_{1,43}$ = 1.59762, P = 0.213
Morph_PC1	Chem_PC1	Grafen	-	Model did not converge
	Chem_PC3			
	Col_PC1			
Morph_PC1	Chem_PC1		137.44	$F_{1,43}$ = 0.69539, P = 0.409
	Chem_PC3			$F_{1,43}$ = 1.31223, P = 0.258
	Col_PC1			$F_{1,43}$ = 1.46459, P = 0.233

Dependent	Independent	BL transform	AIC	
Morph_PC1	Chem_PC3 Col_PC1	OLS	**134.14**	$F_{1,44}$ = 1.63039, P = 0.208 $F_{1,44}$ = 1.80295, P = 0.186
Morph_PC1	Chem_PC3 Col_PC1	PGLS	137.5	$F_{1,44}$ = 1.10603, P = 0.299 $F_{1,44}$ = 1.27298, P = 0.266
Morph_PC1	Chem_PC3 Col_PC1	OU (*d*= 0.270)	135.55	$F_{1,44}$ = 1.48094, P = 0.230 $F_{1,44}$ = 1.57690, P = 0.216
Morph_PC1	Chem_PC3 Col_PC1	G (ρ= 0.232)	135.89	$F_{1,44}$ = 1.49868, P = 0.227 $F_{1,44}$ = 1.47277, P = 0.231
Morph_PC1	Chem_PC3 Col_PC1	P (= 0.274)	136.08	$F_{1,44}$ = 1.52136, P = 0.224 $F_{1,44}$ = 1.45130, P = 0.235
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1 Col_PC2	OLS	144.05	$F_{1,41}$ = 1.96427, P = 0.169 $F_{1,41}$ = 0.19748, P = 0.659 $F_{1,41}$ = 0.17735, P = 0.676 $F_{1,41}$ = 3.57580, P = 0.066 $F_{1,41}$ = 0.02474, P = 0.123
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1 Col_PC2	PGLS	145.49	$F_{1,41}$ = 0.52686, P = 0.472 $F_{1,41}$ = 0.18243, P = 0.671 $F_{1,41}$ = 0.44246, P = 0.509 $F_{1,41}$ = 2.91591, P = 0.095 $F_{1,41}$ = 0.07633, P = 0.931
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1 Col_PC2	OU (*d*=0.402)	145.63	$F_{1,41}$ = 1.12779, P = 0.294 $F_{1,41}$ = 0.21297, P = 0.647 $F_{1,41}$ = 0.30373, P = 0.585 $F_{1,41}$ = 3.27943, P = 0.077 $F_{1,41}$ = 0.04124, P = 0.840
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1 Col_PC2	Using ML	40.38	$F_{1,41}$ = 0.12001, P = 0.731 $F_{1,41}$ = 0.28466, P = 0.596 $F_{1,41}$ = 0.41081, P = 0.525 $F_{1,41}$ = 5.96968, P = 0.019 $F_{1,41}$ = 0.10050, P = 0.753
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1 Col_PC2	P (=0.585)	146.05	$F_{1,41}$ = 0.95285, P = 0.334 $F_{1,41}$ = 0.22411, P = 0.638 $F_{1,41}$ = 0.34745, P = 0.559 $F_{1,41}$ = 3.21922, P = 0.080 $F_{1,41}$ = 0.04466, P = 0.830
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1	OLS	142.08	$F_{1,42}$ = 2.27193, P = 0.139 $F_{1,42}$ = 0.19628, P = 0.660 $F_{1,42}$ = 0.17694, P = 0.676 $F_{1,42}$ = 3.63605, P = 0.063
Morph_PC2	Chem_PC1 Pchem2 Chem_PC3 Col_PC1	PGLS	143.57	$F_{1,42}$ = 0.73445, P = 0.396 $F_{1,42}$ = 0.20199, P = 0.655 $F_{1,42}$ = 0.44858, P = 0.507 $F_{1,42}$ = 2.90372, P = 0.096

Dependent	Independent	BL transform	AIC	
Morph_PC2	Chem_PC1	OU (*d*=0.405	143.66	$F_{1,42}$ = 1.38232, P = 0.246
	Pchem2			$F_{1,42}$ = 0.22190, P = 0.640
	Chem_PC3			$F_{1,42}$ = 0.30524, P = 0.584
	Col_PC1			$F_{1,42}$ = 3.31151, P = 0.076
Morph_PC2	Chem_PC1		38.661	$F_{1,42}$ = 0.36840, P = 0.547
	Pchem2			$F_{1,42}$ = 0.29469, P = 0.590
	Chem_PC3			$F_{1,42}$ = 0.33746, P = 0.564
	Col_PC1			$F_{1,42}$ = 5.89664, P = 0.019
Morph_PC2	Chem_PC1	P (=0.591)	144.08	$F_{1,42}$ = 1.18581, P = 0.238
	Pchem2			$F_{1,42}$ = 0.23147, P = 0.633
	Chem_PC3			$F_{1,42}$ = 0.35179, P = 0.556
	Col_PC1			$F_{1,42}$ = 3.24304, P = 0.079
Morph_PC2	Chem_PC1	OLS	**138.49**	$F_{1,44}$ = 2.31938, P = 0.135
	Col_PC1			$F_{1,44}$ = 3.50084, P = 0.068
Morph_PC2	Chem_PC1	PGLS	140.25	$F_{1,44}$ = 0.78222, P = 0.381
	Col_PC1			$F_{1,44}$ = 2.63644, P = 0.112
Morph_PC2	Chem_PC1	OU (*d*= 0.381)	**140.16**	$F_{1,44}$ = 1.47302, P = 0.231
	Col_PC1			$F_{1,44}$ = 3.10726, P = 0.085
Morph_PC2	Chem_PC1	Grafen	-	Model did not converge
	Col_PC1			
Morph_PC2	Chem_PC1	P (= 0.521)	140.49	$F_{1,44}$ = 1.33058, P = 0.255
	Col_PC1			$F_{1,44}$ = 3.05791, P = 0.087
Morph_PC2	Col_PC1	OU (*d*= 0.415)	168.03	$F_{1,56}$ = 4.47454, P = 0.039
Morph_PC2	Col_PC1		168.09	$F_{1,56}$ = 4.42740, P = 0.040
Morph_PC2	Col_PC1	P (= 0.503)	168.15	$F_{1,56}$ = 4.38405, P = 0.041
Morph_PC3	Chem_PC1	OLS	**111.07**	$F_{1,41}$ = 3.78244, P = 0.059
	Pchem2			$F_{1,41}$ = 9.69415, P = 0.003
	Chem_PC3			$F_{1,41}$ = 0.10806, P = 0.744
	Col_PC1			$F_{1,41}$ = 0.51940, P = 0.475
	Col_PC2			$F_{1,41}$ = 0.36149, P = 0.551
Morph_PC3	Chem_PC1	PGLS	118.82	$F_{1,41}$ = 3.29628, P = 0.077
	Pchem2			$F_{1,41}$ = 0.10209, P = 0.751
	Chem_PC3			$F_{1,41}$ = 0.12787, P = 0.722
	Col_PC1			$F_{1,41}$ = 0.28664, P = 0.595
	Col_PC2			$F_{1,41}$ = 0.05394, P = 0.817
Morph_PC3	Chem_PC1	OU	-	Model did not converge
	Pchem2			
	Chem_PC3			
	Col_PC1			
	Col_PC2			
Morph_PC3	Chem_PC1		113.98	$F_{1,41}$ = 3.96107, P = 0.053
	Pchem2			$F_{1,41}$ = 0.10148, P = 0.752
	Chem_PC3			$F_{1,41}$ = 0.10682, P = 0.745
	Col_PC1			$F_{1,41}$ = 0.53612, P = 0.468
	Col_PC2			$F_{1,41}$ = 0.29857, P = 0.588

Dependent	Independent	BL transform	AIC	
Morph_PC3	Chem_PC1	P (=2.602)	113.07	$F_{1,41}$ = 3.78244, P = 0.059
	Pchem2			$F_{1,41}$ = 9.69415, P = 0.003
	Chem_PC3			$F_{1,41}$ = 0.10806, P = 0.744
	Col_PC1			$F_{1,41}$ = 0.51940, P = 0.475
	Col_PC2			$F_{1,41}$ = 0.36149, P = 0.551
Morph_PC3	Chem_PC1	OLS	213.42	$F_{1,75}$ = 9.40261, P = 0.003
	Pchem2			$F_{1,75}$ = 0.16113, P = 0.689
Morph_PC3	Chem_PC1	PGLS	222.94	$F_{1,75}$ = 5.57214, P = 0.021
	Pchem2			$F_{1,75}$ = 0.15699, P = 0.693
Morph_PC3	Chem_PC1	OU (*d*=0.058)		$F_{1,75}$ = 9.03733, P = 0.004
	Pchem2		199.331	$F_{1,75}$ = 0.15967, P = 0.691
Morph_PC3	Chem_PC1		215.28	$F_{1,75}$ = 9.06034, P = 0.004
	Pchem2			$F_{1,75}$ = 0.16695, P = 0.684
Morph_PC3	Chem_PC1	P (=0.013)	215.42	$F_{1,75}$ = 9.30515, P = 0.003
	Pchem2			$F_{1,75}$ = 0.15996, P = 0.690
Chem_PC1	Morph_PC1	OLS	142.09	$F_{1,41}$ = 0.49775, P = 0.484
	Morph_PC2			$F_{1,41}$ = 1.34250, P = 0.253
	Morph_PC3			$F_{1,41}$ = 1.26752, P = 0.267
	Col_PC1			$F_{1,41}$ = 0.21394, P = 0.646
	Col_PC2			$F_{1,41}$ = 2.66122, P = 0.110
Chem_PC1	Morph_PC1	PGLS	141.5	$F_{1,41}$ = 0.87361, P = 0.355
	Morph_PC2			$F_{1,41}$ = 0.13892, P = 0.711
	Morph_PC3			$F_{1,41}$ = 1.13544, P = 0.293
	Col_PC1			$F_{1,41}$ = 0.01163, P = 0.915
	Col_PC2			$F_{1,41}$ = 3.64323, P = 0.063
Chem_PC1	Morph_PC1	OU (*d*=0.523)	142.2	$F_{1,41}$ = 0.73881, P = 0.395
	Morph_PC2			$F_{1,41}$ = 0.43133, P = 0.515
	Morph_PC3			$F_{1,41}$ = 1.29750, P = 0.261
	Col_PC1			$F_{1,41}$ = 0.08359, P = 0.774
	Col_PC2			$F_{1,41}$ = 3.32512, P = 0.075
Chem_PC1	Morph_PC1		142.45	$F_{1,41}$ = 0.71373, P = 0.403
	Morph_PC2			$F_{1,41}$ = 0.40608, P = 0.527
	Morph_PC3			$F_{1,41}$ = 1.35724 , P = 0.251
	Col_PC1			$F_{1,41}$ = 0.09294, P = 0.762
	Col_PC2			$F_{1,41}$ = 3.35638, P = 0.074
Chem_PC1	Morph_PC1	P =0.644)	142.63	$F_{1,41}$ = 0.69411, P = 0.409
	Morph_PC2			$F_{1,41}$ = 0.39360, P = 0.534
	Morph_PC3			$F_{1,41}$ = 1.39805, P = 0.243
	Col_PC1			$F_{1,41}$ = 0.09859, P = 0.755
	Col_PC2			$F_{1,41}$ = 3.38227, P = 0.073
Chem_PC1	Morph_PC3	OLS	138.14	$F_{1,44}$ = 2.55714, P = 0.117
	Col_PC2			$F_{1,44}$ = 2.68515, P = 0.108

Dependent	Independent	BL transform	AIC	
Chem_PC1	Morph_PC3	PGLS	**136.79**	$F_{1,44}$ = 1.95263, P = 0.169
	Col_PC2			$F_{1,44}$ = 3.69560, P = 0.061
Chem_PC1	Morph_PC3	OU (*d*=0.585)	137.67	$F_{1,44}$ = 2.32702, P = 0.134
	Col_PC2			$F_{1,44}$ = 3.44408, P = 0.070
Chem_PC1	Morph_PC3		137.84	$F_{1,44}$ = 2.39721, P = 0.124
	Col_PC2			$F_{1,44}$ = 3.48624, P = 0.068
Chem_PC1	Morph_PC3	P (=0.699)	137.98	$F_{1,44}$ = 2.44120, P = 0.125
	Col_PC2			$F_{1,44}$ = 3.52945, P = 0.067
Chem_PC1	Col_PC2	OLS	138.79	$F_{1,45}$ = 3.20197, P = 0.080
Chem_PC1	Col_PC2	PGLS	136.83	$F_{1,45}$ = 4.32234, P = 0.043
Chem_PC1	Col_PC2	OU (*d*= 0.690)	138.14	$F_{1,45}$ = 4.14896, P = 0.048
Chem_PC1	Col_PC2		138.37	$F_{1,45}$ = 4.21338, P = 0.046
Chem_PC1	Col_PC2	P (= 0.857)	138.54	$F_{1,45}$ = 4.27644, P = 0.044
Chem_PC2	Morph_PC1	OLS	129.85	$F_{1,41}$ = 0.15917, P = 0.692
	Morph_PC2			$F_{1,41}$ = 0.01707, P = 0.897
	Morph_PC3			$F_{1,41}$ = 5.19487, P = 0.028
	Col_PC1			$F_{1,41}$ = 0.53842, P = 0.467
	Col_PC2			$F_{1,41}$ = 0.41321, P = 0.524
Chem_PC2	Morph_PC1	PGLS	129.92	$F_{1,41}$ = 0.00952, P = 0.923
	Morph_PC2			$F_{1,41}$ = 0.02196, P = 0.883
	Morph_PC3			$F_{1,41}$ = 4.68651, P = 0.036
	Col_PC1			$F_{1,41}$ = 0.46409, P = 0.499
	Col_PC2			$F_{1,41}$ = 0.03311, P = 0.856
Chem_PC2	Morph_PC1	OU (*d*=0.323)	129.91	$F_{1,41}$ = 0.00955, P = 0.922
	Morph_PC2			$F_{1,41}$ = 0.00189, P = 0.892
	Morph_PC3			$F_{1,41}$ = 5.13818, P = 0.028
	Col_PC1			$F_{1,41}$ = 0.57222, P = 0.454
	Col_PC2			$F_{1,41}$ = 0.17822, P = 0.675
Chem_PC2	Morph_PC1		131.05	$F_{1,41}$ = 0.00100, P = 0.975
	Morph_PC2			$F_{1,41}$ = 0.01838, P = 0.893
	Morph_PC3			$F_{1,41}$ = 5.03239, P = 0.030
	Col_PC1			$F_{1,41}$ = 0.55576, P = 0.460
	Col_PC2			$F_{1,41}$ = 0.14249, P = 0.708
Chem_PC2	Morph_PC1	P (=0.811)	131.69	$F_{1,41}$ = 0.00095, P = 0.976
	Morph_PC2			$F_{1,41}$ = 0.01850, P = 0.892
	Morph_PC3			$F_{1,41}$ = 4.86840, P = 0.033
	Col_PC1			$F_{1,41}$ = 0.52294, P = 0.474
	Col_PC2			$F_{1,41}$ = 0.09343, P = 0.761
Chem_PC2	Morph_PC3	OLS	126.06	$F_{1,43}$ = 6.34153, P = 0.016
	Col_PC1			$F_{1,43}$ = 0.57270, P = 0.453
	Col_PC2			$F_{1,43}$ = 0.54726, P = 0.463
Chem_PC2	Morph_PC3	PGLS	**125.97**	$F_{1,43}$ = 5.03003, P = 0.030
	Col_PC1			$F_{1,43}$ = 0.60809, P = 0.440
	Col_PC2			$F_{1,43}$ = 0.00360, P = 0.952

Dependent	Independent	BL transform	AIC	
Chem_PC2	Morph_PC3	OU (*d*= 0.412)	125.95	$F_{1,43}$ = 5.82240, P = 0.020
	Col_PC1			$F_{1,43}$ = 0.69217, P = 0.410
	Col_PC2			$F_{1,43}$ = 0.20234, P = 0.655
Chem_PC2	Morph_PC3		127.07	$F_{1,43}$ = 5.60783, P = 0.022
	Col_PC1			$F_{1,43}$ = 0.68402, P = 0.795
	Col_PC2			$F_{1,43}$ = 0.15530, P = 0.695
Chem_PC2	Morph_PC3	P (= 0.825)	127.71	$F_{1,43}$ = 5.32335, P = 0.026
	Col_PC1			$F_{1,43}$ = 0.65573, P = 0.422
	Col_PC2			$F_{1,43}$ = 0.09697, P = 0.758
Chem_PC2	Morph_PC3	OLS	212.72	$F_{1,76}$ = 0.14509, P = 0.704
Chem_PC2	Morph_PC3	PGLS	212.93	$F_{1,76}$ = 0.13872, P = 0.711
Chem_PC2	Morph_PC3	OU (d= 0.463)	213.18	$F_{1,76}$ = 0.13338, P = 0.716
Chem_PC2	Morph_PC3	Grafen	-	Model did not converge
Chem_PC2	Morph_PC3	Pagel	-	Model did not converge
Chem_PC3	Morph_PC1	OLS	139.05	$F_{1,41}$ = 0.40795, P = 0.527
	Morph_PC2			$F_{1,41}$ = 0.00017, P = 0.989
	Morph_PC3			$F_{1,41}$ = 7.02274, P = 0.011
	Col_PC1			$F_{1,41}$ = 0.76037, P = 0.388
	Col_PC2			$F_{1,41}$ = 0.12232, P = 0.728
Chem_PC3	Morph_PC1	PGLS	145.53	$F_{1,41}$ = 0.23441, P = 0.631
	Morph_PC2			$F_{1,41}$ = 0.01666, P = 0.898
	Morph_PC3			$F_{1,41}$ = 8.31402, P = 0.006
	Col_PC1			$F_{1,41}$ = 0.58588, P = 0.448
	Col_PC2			$F_{1,41}$ = 0.03772, P = 0.847
Chem_PC3	Morph_PC1 Morph_PC2 Morph_PC3 Col_PC1 Col_PC2	OU	-	Model did not converge
Chem_PC3	Morph_PC1		142.42	$F_{1,41}$ = 0.36501, P = 0.549
	Morph_PC2			$F_{1,41}$ = 0.00165, P = 0.968
	Morph_PC3			$F_{1,41}$ = 6.79571, P = 0.013
	Col_PC1			$F_{1,41}$ = 0.7.158, P = 0.402
	Col_PC2			$F_{1,41}$ = 0.1.174, P = 0.734
Chem_PC3	Morph_PC1	P (=2.602)	141.05	$F_{1,41}$ = 0.40795, P = 0.527
	Morph_PC2			$F_{1,41}$ = 0.00017, P = 0.992
	Morph_PC3			$F_{1,41}$ = 7.02274, P = 0.011
	Col_PC1			$F_{1,41}$ = 0.76037, P = 0.388
	Col_PC2			$F_{1,41}$ = 0.12232, P = 0.729
Chem_PC3	Morph_PC1	OLS	144.48	$F_{1,42}$ = 1.50267, P = 0.227
	Morph_PC3			$F_{1,42}$ = 2.04848, P = 0.653
	Col_PC1			$F_{1,42}$ = 1.07453, P = 0.306
	Col_PC2			$F_{1,42}$ = 0.00009, P = 0.992

Dependent	Independent	BL transform	AIC	
Chem_PC3	Morph_PC1	PGLS	143.55	$F_{1,42}$ = 0.26185, P = 0.618
	Morph_PC3			$F_{1,42}$ = 8.87138, P = 0.005
	Col_PC1			$F_{1,42}$ = 0.58540, P = 0.448
	Col_PC2			$F_{1,42}$ = 0.03527, P = 0.852
Chem_PC3	Morph_PC1	OU	-	Model did not converge
	Morph_PC3			
	Col_PC1			
	Col_PC2			
Chem_PC3	Morph_PC1		140.42	$F_{1,42}$ = 0.37453, P = 0.544
	Morph_PC3			$F_{1,42}$ = 7.23602, P = 0.010
	Col_PC1			$F_{1,42}$ = 0.76724, P = 0.386
	Col_PC2			$F_{1,42}$ = 0.11907, P = 0.732
Chem_PC3	Morph_PC1	P (=2.602)	139.05	$F_{1,42}$ = 0.41809, P = 0521
	Morph_PC3			$F_{1,42}$ = 7.43376, P = 0.009
	Col_PC1			$F_{1,42}$ = 0.84558, P = 0.363
	Col_PC2			$F_{1,42}$ = 0.12618, P = 0.724
Chem_PC3	Morph_PC1	OLS	135.19	$F_{1,43}$ = 0.51803, P = 0.476
	Morph_PC3			$F_{1,43}$ = 7.45999, P = 0.009
	Col_PC1			$F_{1,43}$ = 0.82509, P = 0.369
Chem_PC3	Morph_PC1	PGLS	141.59	$F_{1,43}$ = 0.28251, P = 0.598
	Morph_PC3			$F_{1,43}$ = 9.07159, P = 0.004
	Col_PC1			$F_{1,43}$ = 0.56860, P = 0.455
Chem_PC3	Morph_PC1	OU	-	Model did not converge
	Morph_PC3			
	Col_PC1			
Chem_PC3	Morph_PC1		138.56	$F_{1,43}$ = 0.45496, P = 0.504
	Morph_PC3			$F_{1,43}$ = 7.29320, P = 0.009
	Col_PC1			$F_{1,43}$ = 0.74123, P = 0.394
Chem_PC3	Morph_PC1	P (= 2.602)	**137.19**	$F_{1,43}$ = 0.51803, P = 0.476
	Morph_PC3			$F_{1,43}$ = 7.45999, P = 0.009
	Col_PC1			$F_{1,43}$ = 0.82509, P = 0.369
Chem_PC3	Morph_PC3	OLS	211.17	$F_{1,76}$ = 0.16325, P = 0.687
Chem_PC3	Morph_PC3	PGLS	216.05	$F_{1,76}$ = 0.15113, P = 0.698
Chem_PC3	Morph_PC3	OU (*d*= 0.164)	212.98	$F_{1,76}$ = 0.16205, P = 0.688
Chem_PC3	Morph_PC3		213.78	$F_{1,76}$ = 0.15544, P = 0.694
Chem_PC3	Morph_PC3	P (= 0.041)	213.17	$F_{1,76}$ = 0.16085, P = 0.689
Col_PC1	Morph_PC1	OLS	133.59	$F_{1,40}$ = 1.91082, P = 0.174
	Morph_PC2			$F_{1,40}$ = 3.21303, P = 0.081
	Morph_PC3			$F_{1,40}$ = 0.09534, P = 0.759
	Chem_PC1			$F_{1,40}$ = 0.67954, P = 0.414
	Chem_PC2			$F_{1,40}$ = 1.04069, P = 0.314
	Chem_PC3			$F_{1,40}$ = 1.14661, P = 0.291

Dependent	Independent	BL transform	AIC	
Col_PC1	Morph_PC1	PGLS	138.32	$F_{1,40}$ = 0.86753, P = 0.357
	Morph_PC2			$F_{1,40}$ = 2.13593, P = 0.152
	Morph_PC3			$F_{1,40}$ = 0.04323, P = 0.836
	Chem_PC1			$F_{1,40}$ = 0.41897, P = 0.521
	Chem_PC2			$F_{1,40}$ = 1.02934, P = 0.316
	Chem_PC3			$F_{1,40}$ = 0.95998, P = 0.333
Col_PC1	Morph_PC1	OU (*d*=0.199)	135.4	$F_{1,40}$ = 1.60611, P = 0.212
	Morph_PC2			$F_{1,40}$ = 2.92033, P = 0.095
	Morph_PC3			$F_{1,40}$ = 0.0767, P = 0.783
	Chem_PC1			$F_{1,40}$ = 0.64924, P = 0.425
	Chem_PC2			$F_{1,40}$ = 1.09620, P = 0.301
	Chem_PC3			$F_{1,40}$ = 1.09364, P = 0.302
Col_PC1	Morph_PC1		135.22	$F_{1,40}$ = 1.89373, P = 0.176
	Morph_PC2			$F_{1,40}$ = 3.42333, P = 0.071
	Morph_PC3			$F_{1,40}$ = 6.37870, P = 0.802
	Chem_PC1			$F_{1,40}$ = 1.06847, P = 0.307
	Chem_PC2			$F_{1,40}$ = 1.42118, P = 0.240
	Chem_PC3			$F_{1,40}$ = 1.10250, P = 0.290
Col_PC1	Morph_PC1	P (=0.011)	135.59	$F_{1,40}$ = 1.88355, P = 0.178
	Morph_PC2			$F_{1,40}$ = 3.19967, P = 0.081
	Morph_PC3			$F_{1,40}$ = 0.09795, P = 0.756
	Chem_PC1			$F_{1,40}$ = 0.68484, P = 0.413
	Chem_PC2			$F_{1,40}$ = 1.04919, P = 0.312
	Chem_PC3			$F_{1,40}$ = 1.14574, P = 0.291
Col_PC1	Morph_PC1	OLS	131.7	$F_{1,41}$ = 2.05317, P = 0.159
	Morph_PC2			$F_{1,41}$ = 3.50256, P = 0.068
	Chem_PC1			$F_{1,41}$ = 0.60452, P = 0.440
	Chem_PC2			$F_{1,41}$ = 0.98213, P = 0.327
	Chem_PC3			$F_{1,41}$ = 1.10434, P = 0.299
Col_PC1	Morph_PC1	PGLS	136.37	$F_{1,41}$ = 0.92440, P = 0.342
	Morph_PC2			$F_{1,41}$ = 2.30857, P = 0.136
	Chem_PC1			$F_{1,41}$ = 0.38612, P = 0.538
	Chem_PC2			$F_{1,41}$ = 1.08213, P = 0.304
	Chem_PC3			$F_{1,41}$ = 1.02322, P = 0.318
Col_PC1	Morph_PC1	OU (*d*= 0.203)	133.49	$F_{1,41}$ = 1.71536, P = 0.198
	Morph_PC2			$F_{1,41}$ = 3.16631, P = 0.083
	Chem_PC1			$F_{1,41}$ = 0.58830, P = 0.447
	Chem_PC2			$F_{1,41}$ = 1.07783, P = 0.305
	Chem_PC3			$F_{1,41}$ = 1.07906, P = 0.305
Col_PC1	Morph_PC1		133.26	$F_{1,41}$ = 2.11278, P = 0.154
	Morph_PC2			$F_{1,41}$ = 3.78604, P = 0.059
	Chem_PC1			$F_{1,41}$ = 1.18036, P = 0.284
	Chem_PC2			$F_{1,41}$ = 1.63606, P = 0.208
	Chem_PC3			$F_{1,41}$ = 1.10660, P = 0.299

Dependent	Independent	BL transform	AIC	
Col_PC1	Morph_PC1	P (=2.602)	133.7	$F_{1,41}$ = 2.05317, P = 0.159
	Morph_PC2			$F_{1,41}$ = 3.50256, P = 0.068
	Chem_PC1			$F_{1,41}$ = 0.60452, P = 0.441
	Chem_PC2			$F_{1,41}$ = 0.98213, P = 0.327
	Chem_PC3			$F_{1,41}$ = 1.10434, P = 0.299
Col_PC1	Morph_PC1	OLS	130.39	$F_{1,42}$ = 1.86250, P = 0.179
	Morph_PC2			$F_{1,42}$ = 3.09142, P = 0.086
	Chem_PC2			$F_{1,42}$ = 0.83262, P = 0.367
	Chem_PC3			$F_{1,42}$ = 1.16377, P = 0.287
Col_PC1	Morph_PC1	PGLS	134.81	$F_{1,42}$ = 0.79443, P = 0.378
	Morph_PC2			$F_{1,42}$ = 2.19055, P = 0.146
	Chem_PC2			$F_{1,42}$ = 0.92078, P = 0.343
	Chem_PC3			$F_{1,42}$ = 1.14248, P = 0.291
Col_PC1	Morph_PC1	OU (*d*= 0.203)	132.17	$F_{1,42}$ = 1.52391, P = 0.224
	Morph_PC2			$F_{1,42}$ = 2.81966, P = 0.100
	Chem_PC2			$F_{1,42}$ = 0.92056, P = 0.343
	Chem_PC3			$F_{1,42}$ = 1.15425, P = 0.289
Col_PC1	Morph_PC1		132.54	$F_{1,42}$ = 1.63251, P = 0.208
	Morph_PC2			$F_{1,42}$ = 3.01335, P = 0.090
	Chem_PC2			$F_{1,42}$ = 1.00004, P = 0.323
	Chem_PC3			$F_{1,42}$ = 1.13146, P = 0.293
Col_PC1	Morph_PC1	P (= 2.602)	132.39	$F_{1,42}$ = 1.86250, P = 0.180
	Morph_PC2			$F_{1,42}$ = 3.09142 , P = 0.086
	Chem_PC2			$F_{1,42}$ = 0.83262, P = 0.367
	Chem_PC3			$F_{1,42}$ = 1.16377, P = 0.287
Col_PC1	Morph_PC1	OLS	**129.31**	$F_{1,43}$ = 1.58128, P = 0.215
	Morph_PC2			$F_{1,43}$ = 3.08175, P = 0.086
	Chem_PC3			$F_{1,43}$ = 1.10668, P = 0.299
Col_PC1	Morph_PC1	PGLS	133.83	$F_{1,43}$ = 0.73498, P = 0.396
	Morph_PC2			$F_{1,43}$ = 2.17281, P = 0.148
	Chem_PC3			$F_{1,43}$ = 0.99793, P = 0.323
Col_PC1	Morph_PC1	OU (*d*= 0.181)	**131.15**	$F_{1,43}$ = 1.34568, P = 0.252
	Morph_PC2			$F_{1,43}$ = 2.83026, P = 0.099
	Chem_PC3			$F_{1,43}$ = 1.08234, P = 0.304
Col_PC1	Morph_PC1		131.68	$F_{1,43}$ = 1.30931, P = 0.259
	Morph_PC2			$F_{1,43}$ = 2.92590, P = 0.094
	Chem_PC3			$F_{1,43}$ = 1.05561, P = 0.310
Col_PC1	Morph_PC1	P (= 2.602)	131.31	$F_{1,43}$ = 1.58128, P = 0.215
	Morph_PC2			$F_{1,43}$ = 3.08175, P = 0.086
	Chem_PC3			$F_{1,43}$ = 1.10668, P = 0.299
Col_PC1	Morph_PC2	OLS	164.47	$F_{1,56}$ = 5.16943, P = 0.027
Col_PC1	Morph_PC2	PGLS	171.36	$F_{1,56}$ = 3.96806, P = 0.053

Dependent	Independent	BL transform	AIC	
Col_PC1	Morph_PC2	OU (*d*= 0.062)	166.45	$F_{1,56}$ = 5.06174, P = 0.028
Col_PC1	Morph_PC2		166.16	$F_{1,56}$ = 5.38966, P = 0.024
Col_PC1	Morph_PC2	P (= 2.602)	166.47	$F_{1,56}$ = 5.16943, P = 0.027
Col_PC2	Morph_PC1	OLS	138.86	$F_{1,40}$ = 1.40708, P = 0.242
	Morph_PC2			$F_{1,40}$ = 0.00421, P = 0.948
	Morph_PC3			$F_{1,40}$ = 0.42235, P = 0.519
	Chem_PC1			$F_{1,40}$ = 2.33848, P = 0.134
	Chem_PC2			$F_{1,40}$ = 0.10166, P = 0.751
	Chem_PC3			$F_{1,40}$ = 0.06797, P = 0.796
Col_PC2	Morph_PC1	PGLS	145.48	$F_{1,40}$ = 0.71845, P = 0.402
	Morph_PC2			$F_{1,40}$ = 0.01547, P = 0.902
	Morph_PC3			$F_{1,40}$ = 0.04638, P = 0.831
	Chem_PC1			$F_{1,40}$ = 3.89046, P = 0.055
	Chem_PC2			$F_{1,40}$ = 0.16941, P = 0.683
	Chem_PC3			$F_{1,40}$ = 0.00062, P = 0.980
Col_PC2	Morph_PC1	OU (*d*=0.012)	140.86	$F_{1,40}$ = 1.39872, P = 0.244
	Morph_PC2			$F_{1,40}$ = 0.00402, P = 0.949
	Morph_PC3			$F_{1,40}$ = 0.41677, P = 0.522
	Chem_PC1			$F_{1,40}$ = 2.35168, P = 0.133
	Chem_PC2			$F_{1,40}$ = 0.09704, P = 0.330
	Chem_PC3			$F_{1,40}$ = 0.06779, P =0.796
Col_PC2	Morph_PC1	Using ML	35.11	$F_{1,40}$ = 2.54479, P = 0.118
	Morph_PC2			$F_{1,40}$ = 0.06992, P = 0.793
	Morph_PC3			$F_{1,40}$ = 1.76297, P = 0.192
	Chem_PC1			$F_{1,40}$ = 6.33014, P = 0.016
	Chem_PC2			$F_{1,40}$ = 0.13748, P = 0.713
	Chem_PC3			$F_{1,40}$ = 0.31106, P = 0.580
Col_PC2	Morph_PC1	P (=2.602)	140.86	$F_{1,40}$ = 1.40708, P = 0.242
	Morph_PC2			$F_{1,40}$ = 0.00422, P = 0.949
	Morph_PC3			$F_{1,40}$ = 0.42235, P = 0.519
	Chem_PC1			$F_{1,40}$ = 2.33848, P = 0.134
	Chem_PC2			$F_{1,40}$ = 0.10166, P = 0.752
	Chem_PC3			$F_{1,40}$ = 0.06798, P = 0.797
Col_PC2	Morph_PC1	OLS	133.03	$F_{1,43}$ = 1.65919, P = 0.205
	Morph_PC3			$F_{1,43}$ = 0.30215, P = 0.585
	Chem_PC1			$F_{1,43}$ = 2.96620, P = 0.092
Col_PC2	Morph_PC1	PGLS	139.71	$F_{1,43}$ = 0.75547, P = 0.390
	Morph_PC3			$F_{1,43}$ = 0.23235, P = 0.632
	Chem_PC1			$F_{1,43}$ = 4.03518, P = 0.051
Col_PC2	Morph_PC1	OU (d= 0.011)	135.03	$F_{1,43}$ = 1.64759, P = 0.206
	Morph_PC3			$F_{1,43}$ = 0.30013, P = 0.587
	Chem_PC1			$F_{1,43}$ = 2.97553, P = 0.092

Dependent	Independent	BL transform	AIC	
Col_PC2	Morph_PC1		34.18	$F_{1,43}$ = 2.78026, P = 0.103
	Morph_PC3	Using ml		$F_{1,43}$ = 3.58833, P = 0.065
	Chem_PC1			$F_{1,43}$ = 8.18401, P = 0.006
Col_PC2	Morph_PC1	P (= 2.602)	135.03	$F_{1,43}$ = 1.65919, P = 0.205
	Morph_PC3			$F_{1,43}$ = 0.30215, P = 0.585
	Chem_PC1			$F_{1,43}$ = 2.96620, P = 0.092
Col_PC2	Morph_PC1	OLS	131.36	$F_{1,44}$ = 1.46987, P = 0.232
	Chem_PC1			$F_{1,44}$ = 3.68982, P = 0.061
Col_PC2	Morph_PC1	PGLS	137.96	$F_{1,44}$ = 0.65749, P = 0.422
	Chem_PC1			$F_{1,44}$ = 4.72625, P = 0.035
Col_PC2	Morph_PC1	OU (*d*= 0.017)	133.36	$F_{1,44}$ = 1.45371, P = 0.234
	Chem_PC1			$F_{1,44}$ = 3.70301, P = 0.061
Col_PC2	Morph_PC1		131.05	$F_{1,44}$ = 1.30740, P = 0.259
	Chem_PC1			$F_{1,44}$ = 7.42191, P = 0.009
Col_PC2	Morph_PC1	P (= 2.602)	133.36	$F_{1,44}$ = 1.46987, P = 0.232
	Chem_PC1			$F_{1,44}$ = 3.68982, P = 0.061
Col_PC2	Chem_PC1	OLS	130.9	$F_{1,45}$ = 3.20197, P = 0.080
Col_PC2	Chem_PC1	PGLS	136.65	$F_{1,45}$ = 4.32234, P = 0.043
Col_PC2	Chem_PC1	OU (*d*= 0.061)	132.89	$F_{1,45}$ = 3.26851, P = 0.077
Col_PC2	Chem_PC1		**130.44**	$F_{1,45}$ = 6.51240, P = 0.014
Col_PC2	Chem_PC1	P (= 2.602)	132.9	$F_{1,45}$ = 3.20197, P = 0.080

Appendix B Pearson's correlation on morphology of vertebrate-dispersed fruits from Cardoso Island, Atlantic rainforest, Brazil.

	RY	Pulp/ Seed	Seed number	Seed Mass	Seed length	Seed diam.	Pulp dry mass	Pulp fresh mass	Fruit fresh mass	Fruit length
Fruit diameter	-0.02	0.15	0.24*	0.72**	0.40**	0.47**	0.89**	0.97**	0.97**	0.90**
Fruit length	-0.01	0.02	0.17	0.79**	0.52**	0.52**	0.88**	0.90**	0.95**	
Fruit fresh mass	0.00	0.08	0.19	0.79**	0.48**	0.53**	0.93**	0.97**		
Pulp fresh mass	0.00	0.21*	0.25*	0.68**	0.37**	0.42**	0.90**			
Pulp dry mass	0.37**	0.24	0.19*	0.75**	0.44**	0.48**				
Seed diameter	-0.02	-0.47*	-0.67**	0.76**	0.90**					
Seed length	-0.04	-0.47**	-0.64**	0.73**						
Seed mass	0.03	-0.46**	-0.16							
Seed number	0.06	0.49**								
Pulp/seed	0.45**									

*P<0.01,**P<0.001

Appendix C Pearson's correlation on nutritional traits of vertebrate-dispersed fruits from Cardoso Island, Atlantic rainforest, Brazil.

	Tannins	Phenol	Water	Energy	Sugar	Protein
Lipids	-0.13	0.08	-0.52**	0.97**	-0.47**	0.23*
Proteins	-0.29*	-0.02	0.13	0.35*	-0.22	
Sugar	0.06	-0.17	0.28*	-0.44**		
Energy	-0.18	0.03	-0.50**			
Water	-0.11	-0.1				
Phenol	0.69**					

*P<0.01, **P<0.001

Appendix D Pearson's correlation on the three variables that characterize the reflectance spectra (brightness, chroma, and hue) and color chromatic and achromatic contrasts according to bird and primate eye models from vertebrate-dispersed fruits from Cardoso Island, Atlantic rainforest, Brazil.

	Chroma	Hue	Brightness	Achromatic bird	Chromatic bird	Achromatic primate
Chromatic primate	0.19	-0.40*	-0.26*	0.42*	0.82**	0.81**
Achromatic primate	0.17	-0.26	-0.45**	0.81**	0.57**	
Chromatic bird	0.29*	-0.39*	-0.30*	0.40*		
Achromatic bird	0.09	-0.25	-0.48**			
Brightness	-0.29*	0.36*				
Hue	-0.17					

*P<0.01, **P<0.001

Capítulo 2

Why are fruits colorful? The relative importance of achromatic and chromatic contrasts for detection by birds

"Let the fruit be ever so luscious and ever so laden with sweet syrups, it can never secure the suffrages of the higher animals if it lies hidden beneath a mass of green foliage, or clothes itself in the quite garb of the retiring nut. To attract from a distance the eyes of wandering birds or mammals, it must dress itself up in a gorgeous livery of crimson, scarlet and orange".

(Allen 1879)

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ORIGINAL PAPER

Why are fruits colorful? The relative importance of achromatic and chromatic contrasts for detection by birds

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Abstract The colors of fruits and flowers are traditionally viewed as an adaptation to increase the detectability of plant organs to animal vectors. The detectability of visual signals increases with increasing contrasts between target and background. Contrasts consist of a chromatic aspect (color) and an achromatic aspect (light intensity), which are perceived separately by animals. To evaluate the relative importance of fruits' chromatic and achromatic contrasts for the detection by avian fruit consumers we conducted an experiment with artificial fruits of four different colors in a tropical forest. We displayed the fruits against two different backgrounds, an artificial background and a natural one, because they differed in achromatic properties. We found no effect of the type of background on fruit detection rates. Detection rates differed for the four fruit colors. The probability of detection was explained by the chromatic contrast between fruits and their background, not by the achromatic contrasts. We suggest that birds attend primarily to chromatic contrast probably because these are more reliably detected under variable light conditions. Consistent with this hypothesis, we found habitat-specific differences in the conspicuousness of natural fruit colors in the study area. Fruits of understory species that are subjected to the variable light conditions within a forest displayed higher chromatic contrasts than species growing in the open restinga forest with constant bright illumination. There was no such difference for achromatic contrasts. In sum, we suggest that fruit colors differ between habitats because fruit colors that have strong chromatic contrasts against background can increase plants' reproductive success, particularly under variable light conditions.

Keywords Atlantic forest · Eye model · Frugivory · Plant–animal interaction · Signal

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Introduction

Plants that rely on color-sensitive animals to pollinate their flowers and disperse their seeds are expected to increase their reproductive success with conspicuous colors that facilitate detection and attract animal vectors (Kerner 1895; Schaefer et al. 2004). The colors of their reproductive organs therefore meet the criteria of classical signals, which are defined as structures that increase the fitness of the sender by altering the behavior of other organisms such as seed dispersers as receivers (Maynard-Smith and Harper 1995). Although fruit colors are traditionally viewed as an adaptation to seed dispersers, the selective pressures on fruit coloration are not well understood (Willson and Whelan 1990; Schmidt et al. 2004). During the 1980s and early 1990s, the most influential hypothesis aimed to explain how seed dispersers might influence the evolution of plant coloration assumed that they have strong preferences for certain colors. Although some studies reported color preferences of fruit consumers (Puckey et al. 1996; Siitari et al. 1999; Whitney 2005), most bird species exhibit inconsistent and transient color choices with high variability within and between individuals (Willson et al. 1990; Willson and Comet 1993; Traveset and Willson 1998; Schmidt et al. 2004).

In general, the detectability of a visual signal is determined by its contrast against background, the visual conditions during signaling and by the visual perception of the animal receiving the signal (Endler 1990). In recent years, eye models that account for the spectral sensitivities of animals (Vorobyev and Osorio 1998) are increasingly used to predict the optimal design of signals in evolutionary ecology (Endler and Mielke 2005). Animals can use different aspects of a signal to detect and discriminate objects (Giurfa et al. 1997). Under constant conditions in the laboratory, birds and insects use chromatic aspects of color for the detection of large targets and achromatic aspects (that are based solely on differences in the intensity of reflected light), for the detection of small objects and pattern (Osorio et al. 1999; Spaethe et al. 2001). However, eye models make no predictions on the relative importance of chromatic and achromatic aspects of a signal and they do not predict detectability if the intensity of illuminating light varies or the target differs in distance to the receiver (Vorobyev and Osorio 1998). To assess optimal signal design, it is therefore important to test detection rates of differently colored fruits under natural conditions with variable illumination.

We conducted an experiment with artificial fruits in the understory of a tropical lowland forest and determined fruit detection by birds. To study which aspects of a signal influence fruit detection, we designed four differently colored artificial fruits and displayed them against two different backgrounds in a 4×2 design. We used an avian eye model (Vorobyev and Osorio 1998) to determine chromatic and achromatic contrasts between fruits and their background as seen by birds. Because the backgrounds sported very different achromatic properties, the experiment allowed us to evaluate the relative importance of chromatic and achromatic contrasts for fruit detection. We also analyzed the fruit-foliage contrasts of natural fruit species that grow in two different forest types, the lowland forest characterized by drastic changes in illumination between sun spots and dark forest shade and the open restinga forest (shrubland) by relatively constant ambient light. We expected that species growing under different light conditions differ in their fruit-foliage contrasts with higher contrasting fruits in the forest with lower light intensity and more variability in ambient light.

Materials and methods

Study site

Fieldwork was carried out from July to August 2004 on Ilha do Cardoso State Park, a subtropical island, in São Paulo state, southeastern Brazil (25°05′ S; 47°53′ W). The vegetation of the island is composed exclusively of Atlantic rain forest and is constituted by five different types: mangroves, dune vegetation, restinga forest (shrubland), lowland tropical rainforest and highland tropical rainforest (Noffs and Baptista Noffs 1982). The present study was conducted in the lowland tropical rainforest, which is characterized by an understory level and a relatively continuous and dense canopy level that can reach 20 m with a large amount of epiphytes and vines. The adjacent restinga forest resides on sandy soils and is characterized by an open canopy that can reach 4–5 m (Bernardi et al. 2005). The most important frugivorous birds in the forest understory are *Chyroxiphia caudata* (Pipridae), *Turdus rufiventris*, *Turdus albicolis* (Turdidae) and tanagers (Thraupidae), such as *Tangara* and *Tachyphonus* (Marsden et al. 2003).

The climate is generally warm and wet throughout the year but may be divided into two seasons: a cold and drier period from April to August when temperature may drop to nearly 13°C and rainfall is ~500 mm, and a warm and rainier period from September to March when temperature may reach 32°C and rainfall 1800 mm (Oliveira-Filho and Fontes 2000).

Experimental design

We used modeling clay (plasticine) to shape spherical artificial fruits of 14 mm diameter that have been used in previous studies on fruit detection by birds (see Alves-Costa and Lopes 2001; Galetti et al. 2003). In the lowland forest, we placed fruits in 240 shrubs (60 for each different color) at 50 m distance from each other. In each shrub we attached to branches 20 single fruits of the same color with a string. Only shrubs between 1 and 2 m height without fruits or flowers were selected for the experiment. Previous experiments on captive frugivorous birds showed that they promptly accepted the artificial fruits (M.G. unpublished data).

We selected four different fruit colors: UV-blue, red, black and white. We chose these colors because their chromatic and achromatic contrasts closely matched those of natural fruits (Fig. 1), except for the achromatic contrasts of white fruits. Our artificial fruits are thus a good representation of natural variation in fruit contrasts at our study site. We used two types of backgrounds, one artificial background consisting of 50 mm circles of yellow cardboard that were attached with the fruits. A slit and central hole allowed the artificial background to be placed with the same string of the artificial fruit and this close association between fruit and background permitted birds to view and detect fruits consistently against this background even if seen from different angles. This background was considerably larger than fruits and larger than conspicuously colored secondary structures that are associated with fruit displays (e.g., bracts; Burns and Dalen 2002). The artificial background was selected because it differed strongly from natural backgrounds in achromatic contrasts but yielded similar chromatic contrast (see below). Every second shrub that we used for the experiments featured these artificial backgrounds. The remaining displays (50%) had no artificial background and hung in front of natural backgrounds i.e. the leaves. We used a random design in our experiment so that color and presence or absence of background were randomly selected. Fruits were checked after 96 h, by counting the

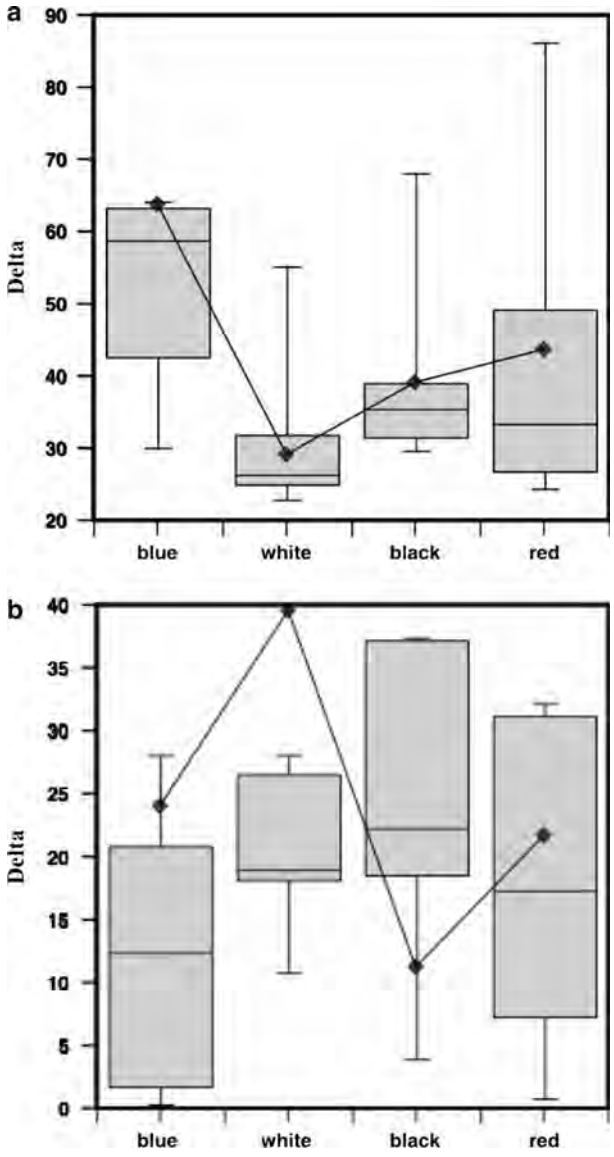

Fig. 1 Mean and standard deviation of natural (**a**) chromatic and (**b**) achromatic contrasts (jnds values) of blue, red, black and white fruits in the study site. The line illustrates the contrasts of artificial fruits

number of pecked or removed fruits. Different animals leave different marks in the artificial fruits, which made it possible to distinguish between mammals (teeth mark), insects (dots or stripes) and birds (beak mark) (Alves-Costa and Lopes 2001). Marks of mammals and insects were ignored. Because teeth marks were recorded in very few cases (two fruits), we assumed that birds removed all missing fruits.

Color measurements and contrast calculation

We measured the reflectance spectra of 15 artificial fruits of each color, 15 artificial backgrounds and leaves of the 16 most common shrubs in the study site as an

approximation of natural foliage background. In order to account for variability in leaf coloration within species, we measured 10 leaves of these 16 shrubs and calculated the mean reflectance of each species. We computed the mean of the 16 species as an approximation of overall natural background reflectance (Fig. 2). We also measured the reflectance of 20 natural fruits and 10 leaves of 16 common shrubs in the lowland forest and of 20 fruits and 10 leaves of 34 species of the restinga forest. We performed all measurements with Ocean optics USB2000 spectrometer and a Top Sensor System Deuterium-Halogen DH-2000 (both Ocean Optics, Duiven, The Netherlands) as a standardized light source. Reflectance was measured as the proportion of a standard white reference tile (Top Sensor Systems WS-2).

Chromatic and achromatic contrasts between the mean reflectance of fruits and artificial background or leaves were calculated according to the model of avian vision, which assumes that receptor noise limits discrimination (Vorobyev and Osorio 1998). Because passerine birds are the most important frugivorous birds in the understory of our study site (see above) and of neotropical forests in general (Loiselle and Blake 1991), we used an eye model based on the spectral sensitivities of the blue tit (*Cyanistes caeruleus*) with a UVS cone (Hart et al. 2000). Based on analytical approximation of cone visual pigments and oil droplet spectra, we calculated the quantum catch of each class of single cones (LWS, MWS, SWS, UVS), denoted by the subscript *i*, as the integrated product of the receptor sensitivity spectrum (R*i*), reflectance spectrum (S), and illumination spectrum (I):

$$Q_i = R_i(\lambda)S(\lambda)I(\lambda)d\lambda \quad (1)$$

The quantum catches are used to find relative contrasts against fruits and background as the log of the quotient of quantum catches from both spectra. The result of this calculation is the contrast Δf for each receptor type *i*:

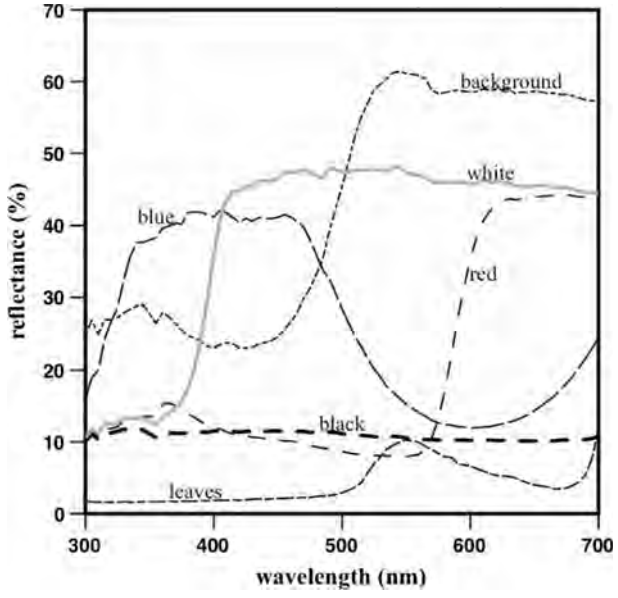

Fig. 2 Mean reflectance spectra of blue, red, black, white artificial fruits, artificial background and leaves of the understory

$$\Delta f_i = \ln(Q_i \text{ fruit}) - \ln(Q_i \text{ background}) = \ln(Q_i \text{ fruit}/Q_i \text{ background}) \quad (2)$$

To quantify discrimination using all receptor types in a given visual system, each receptor class is first assigned a noise value ω based on its individual Weber fraction (v) and on the receptor proportion (n) (Vorobyev et al. 2001):

$$\omega_i = v_i/n_i \quad (3)$$

Then we calculated discrimination values for tetrachromatic visual system. The subscript number of each variable in Eq. 4 is the value given for a particular receptor class:

$$\begin{aligned}\Delta S^2 = {} & [(\omega_1\omega_2)^2(\Delta f_4 - \Delta f_3)^2 + (\omega_1\omega_3)^2(\Delta f_4 - \Delta f_2)^2 \\ & + (\omega_1\omega_4)^2(\Delta f_3 - \Delta f_2)^2 + (\omega_2\omega_3)^2(\Delta_4 - \Delta f_1)^2 \\ & + (\omega_2\omega_4)^2(\Delta f_3 \Delta f_1)^2 + (\omega_3\omega_4)^2(\Delta_2 - \Delta f_1)^2]/[(\omega_1\omega_2\omega_3)^2 \\ & + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2]\end{aligned} \quad (4)$$

Results of the calculation using Eq. 4 provide the chromatic distance (ΔS) separating the perceptual values of two spectra in receptor space. The units for ΔS are jnds (just noticeable differences), where 1 jnd is at the threshold of discrimination, values less than 1 jnd indicate that two colors are indistinguishable and values above 1 can be discriminated (Osorio and Vorobyev 1996).

The achromatic (brightness contrast) analysis is similar to the chromatic one, where comparisons are based on brightness differences alone:

$$\Delta S = |\Delta f_i/\omega|$$

Fruit colors differed in their chromatic contrasts against natural and artificial background (one-way ANOVA, $F = 11.61$, df = 3, $P < 0.0001$; $F = 738.91$, df = 3, $P < 0.0001$, respectively). UV-blue fruits had higher contrasts than black and white fruits, and red fruits had higher contrasts than white fruits against leaves. Against artificial background, all fruits differed in their contrasts (UV-blue > red > black > white) (Table 1, 2). Fruits also differed in their achromatic contrasts against leaves and the artificial background (one-way ANOVA, $F = 21.64$, df = 3, $P < 0.0001$, $F = 4.42$, df = 3, $P = 0.01$, respectively). Against leaves, black fruits had smaller contrasts than blue and white fruits. Against artificial background, all fruits differed in their contrasts, excepted for blue and red (Tables 1, 2). Fruits were darker than the artificial background and brighter than leaves (see signs in Table 1).

In statistical analyses we used detection as a binary response variable. In other words, we analyzed whether or not a shrub with artificial fruits was detected after 96 h. When a shrub had at least one pecked or removed fruit we consider that birds detected it, otherwise it counted as not detected. We used detection as the dependent variable and chromatic and achromatic contrast (values of jnd) as independent variables and the presence or absence of the artificial background as a fixed factor in Logistic Regression.

Results

After 96 h, 68.3% of all shrubs were detected by birds. Nearly half (48.8%) of all detected shrubs had artificial backgrounds and 51.2% of shrubs had no background. Thus, although the artificial background contrasted against the leaves (chromatic contrasts 17 jnds,

Table 1 Chromatic and achromatic contrasts (mean values of jnds) of fruit color against natural and artificial background

Color	Chromatic		Achromatic	
	Artificial	Natural	Artificial	Natural
Blue	50.11	63.72	(–)18.82	(+) 24.01
Red	30.77	43.64	(–)21.19	(+) 21.64
Black	23.83	39.04	(–)31.64	(+) 11.19
White	17.02	29.09	(–)3.29	(+) 39.54

For achromatic contrasts, positive and negative values indicate whether fruit is darker or brighter than the background

Table 2 Results of the post hoc test (*P*-values) following ANOVA, from multiple comparisons between fruit colors against both backgrounds (natural and artificial) in relation to chromatic and achromatic contrasts

Multiple comparisons		Chromatic		Achromatic	
		Natural	Artificial	Natural	Artificial
Blue	Red	0.5	0.003	0.9	0.9
	Black	0.001	<0.001	<0.001	0.03
	White	<0.001	0.002	0.01	0.9
Red	Black	0.05	<0.001	<0.001	0.1
	White	<0.001	<0.001	0.002	0.7
Black	White	0.4	<0.001	<0.001	0.01

The mean difference is significant at the 0.05 level

achromatic contrasts 12 jnds), its presence did not influence the likelihood of detection by frugivorous birds ($\chi^2 = 0.02$, $P = 0.87$). Supporting the observation that the artificial background did not influence detection rate, we found no difference between the total number of pecked fruits per shrub between shrubs with and without artificial background (one-way ANOVA, $F = 0.004$, df = 1, $P = 0.94$).

Detection rates of the four fruit colors differed. The probability of detection was explained by the chromatic contrast between fruits and their artificial background ($\chi^2 = 4.72$, df = 1, $P = 0.02$) and natural background ($\chi^2 = 33.22$, df = 1, $P < 0.0001$). Fruits with higher chromatic contrasts had higher rates of detection with a strong correlation between the percentage of detected shrubs and their chromatic contrasts ($r_s = 1.0$, $P < 0.001$) (Fig. 3).

Although the results was also significant for achromatic contrasts against artificial and natural background ($\chi^2 = 4.69$, df = 1, $P = 0.03$; $\chi^2 = 11.75$, df = 1, $P < 0.001$, respectively), there was no correlation between the percentage of detected shrubs and their achromatic contrasts ($r_s = –0.2$, $P = 0.8$) (Fig. 3). The result predicted an unrealistic inverse relationship between achromatic contrast and detection that did not provide a close match of the variation in detection rates (see Fig. 3).

Chromatic contrasts of natural fruits against leaves from common understory species of the lowland forest differed from those of the restinga forest. Higher values of contrasts were found in the lowland forest (45 jnds ± 3.3 (mean ± SE) in comparison with restinga

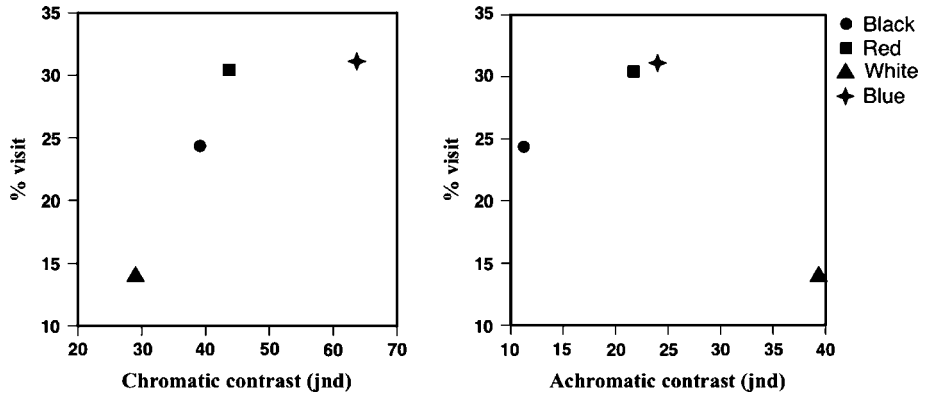

Fig. 3 Relationship between total percentage of detection and chromatic and achromatic contrasts of fruits against natural background (values of jnds)

forest (34 jnds ± 2.4 (mean ± SE) (t = 2.60, P = 0.01) (Fig. 4). Achromatic contrasts of natural fruits did not differ between lowland species (19 jnds ± 2.4; mean ± SE) and restinga species (17 jnds ± 3.5) (t = 0.52, P = 0.60) (Fig. 4).

Discussion

We found that patterns of fruit detection in the forest understory are mediated by chromatic contrasts. Achromatic contrasts do not explain fruit detection because higher achromatic contrasts did not result in higher probability of detection. Also, the inverse relationship between achromatic contrast and detection that the results of our experiment suggest is unlikely to explain general patterns of detection probabilities.

Our result that chromatic contrasts influence fruit detection by birds is based on the detection rates of only four different colors. Testing detection rate of a larger range of colors might yield different results. However, there are different lines of evidence for why we consider this possibility unlikely. First, in the lone experiment on this subject Schaefer et al. (2006) found that—similar to our results—crows prioritized chromatic over achromatic contrasts when searching for fruits among foliage. Second, our comparison between habitats, albeit restricted to two habitats, showed that fruits displayed in different illumination differed in their chromatic contrasts but not in their achromatic contrasts. Alternatively, fruit detection in our study might have been influenced by pre-existing color preferences rather than by fruit perception. However, studies that analyzed fruit consumption as a function of fruit colors and contrasts concluded that fruit removal is a function of contrasts and not of color per se (Schmidt et al. 2004; Schaefer et al. 2006). This conclusion is consistent with the generally inconsistent and transient color preferences of most frugivorous birds that are characterized by high inter- and intra-specific variability (Willson et al. 1990; Wilson and Comet 1993; Traveset and Wilson 1998; Schmidt et al. 2004). Finally, chromatic and achromatic contrasts of artificial fruits generally matched those of natural fruits at the study site, thus, we assume that chromatic contrasts rather than color preferences and pre-existing biases explain the patterns of fruit detection in our study.

We suggest that birds rely on chromatic contrast for fruit detection, probably because variation in chromatic composition of illumination is smaller than variation in achromatic

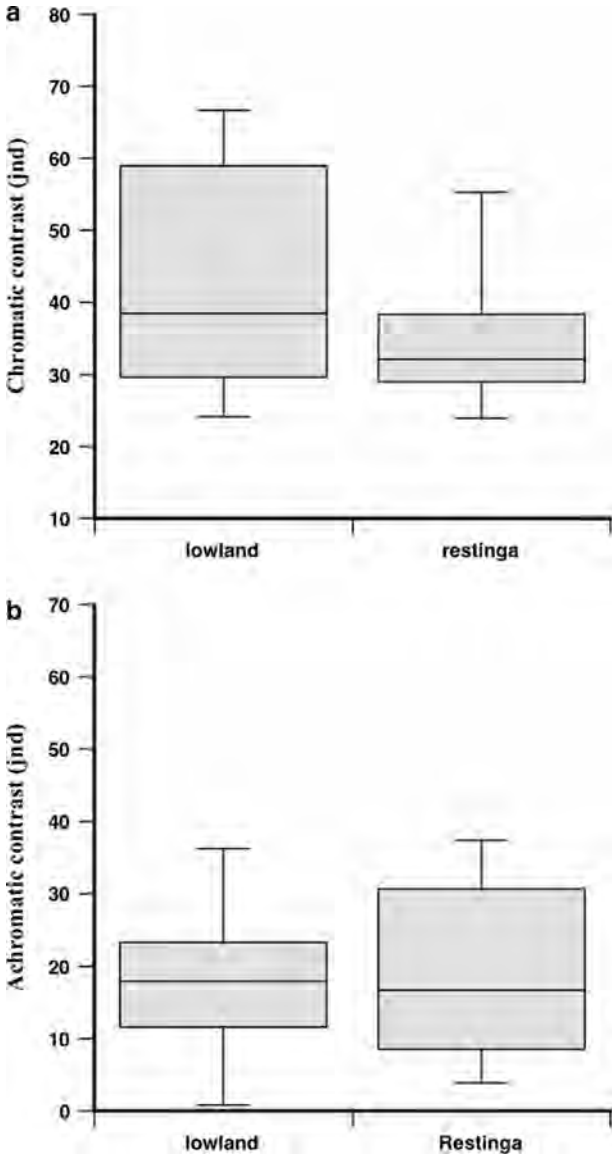

Fig. 4 Illustrated are the contrasts of natural fruits in the lowland forest (N = 16) and restinga forest (N = 34). (**a**) Chromatic contrasts (jnds) are higher in fruit species of the lowland forest than the restinga forest, (**b**) and achromatic contrasts (jnds) are similar between both forest types. Illustrated are medians, mid-quartiles, 90th and 10th percentiles

composition and consequently chromatic contrasts might be more reliable cues under changing light conditions (Troost 1998; Kelber 2005). This logic is especially applicable to the task of fruit detection against a background of foliage, because foliage consists of high achromatic variation owing to alternating patterns of sun spots and shadows. Analyzing color perception by frugivores primates, Summer and Mollon (2000) discussed the

importance of this effect for fruit detection. They concluded that achromatic fruit signals are difficult to detect for primates because of large achromatic variance in foliage.

In our experiment, fruit detection was not influenced by the presence of an artificial background. On the first glance this result is surprising because it is generally believed that the secondary structure associated with fruit display increases the consumption rates of frugivores birds (Morden-Moore and Willson 1982; Wheelwright and Janson 1985; Whelan and Wilson 1994). In our study the artificial background reduced the chromatic contrast between fruits and background to lower values compared to those against leaves but the background itself also contrasted with leaves. There are several possibilities to explain why the artificial background had no effect on detection. First, the reduction in chromatic contrasts was not strong enough to influence patterns of detection. In other words, contrasts between artificial fruits and artificial background were still high enough to warrant detection. Second, the artificial background we used was not big enough to affect the likelihood of detection. We consider this possibility unlikely given that our background was larger than the secondary structures that plants use to increase detection rates by birds (Burns and Dalen 2002). Lastly, the lack of influence of the artificial background might be explicable because its shape differed from that of plants' secondary structures and birds might not have associated this background with fruit rewards.

The model developed by Vorobyev and Osorio (1998) assumes that discrimination can be made quickly and under increasingly unfavorable conditions as values of "jnd" becomes higher than 1. The model is based on the receptor noise of the four cone types. It expects that all objects with values above 10 jnds are always detected because higher levels of noise are not physiologically plausible. The model fits with behavioral evidence in controlled settings in the laboratory (Maier 1992). We found that detection increased with contrasts (from white to black to red fruits) and that this relationship tends to become stable for values above 40 jnds (i.e., no increase in detection rates from red to UV-fruits). In visual searches the likelihood of detection will increase asymptotically until a target is so conspicuous that it will always be detected. The asymptotic curve resulting from our experiment (Fig. 1) might well describe detection thresholds for birds under variable light conditions, although we acknowledge that the conditions and the scope of our experiment are likely too limited to infer general patterns of fruit detection. Importantly, however, our results show that in natural conditions where light intensity and the distance between observer and prey vary, higher contrasts might increase the chances of detection. We emphasize that this applies particularly to the forest understory where rare but regular sun spots might be important distractors for visual searches.

Consistent with the importance of ambient light conditions on fruit detection by birds, we found that natural understory fruits of the lowland forest had higher chromatic contrasts but similar achromatic contrasts than fruit species growing in neighboring areas of restinga forest with bright illumination. To the best of our knowledge, this is the first broad comparative study showing that fruit colors differ between habitats and that this differentiation is likely linked to fruit detection rates by seed dispersers. Although this result is based on comparing two sites only, it fits the observation made by Summer and Mollon (2000) that understory fruits tend to have higher contrasts than fruits in the canopy. Based on our data we suggest that it is particularly important to maximize color chromatic contrasts for animal-dispersed species growing inside the forest under a relatively closed canopy with low light intensity.

In sum, this is the first work that evaluated the importance of chromatic and achromatic signals in fruit detection by birds in natural conditions. We concluded that birds attend primarily to chromatic contrasts probably because they are a more reliable signal under

changing light conditions. Thus, chromatic contrasts play an important role in fruit frugivore interaction by increasing the chances of fruit detection. Plants might therefore increase removal rates and, indirectly, their reproductive success by displaying fruit colors with strong chromatic contrasts against background.

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Capítulo 3

Does attraction to frugivores or defense against pathogens shape fruit pulp composition?

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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Does attraction to frugivores or defense against pathogens shape fruit pulp composition?

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Abstract Fruit traits evolve in response to an evolutionary triad between plants, seed dispersers, and antagonists that consume fruits but do not disperse seeds. The defense trade-off hypothesis predicts that the composition of nutrients and of secondary compounds in fruit pulp is shaped by a trade-off between defense against antagonists and attraction to seed dispersers. The removal rate model of this hypothesis predicts a negative relationship between nutrients and secondary compounds, whereas the toxin-titration model predicts a positive relationship. To test these alternative models, we evaluated whether the contents of nutrients and secondary compounds can be used to predict fruit removal by mutualists and pathogens in 14 bird-dispersed plants on a subtropical island in São Paulo state, southeastern Brazil. We selected eight to ten individuals of each species and prevented fruit removal by covering four branches with a net and left fruits on four other branches available to both, vertebrate fruit consumers and pathogens. The persistence of ripe fruits was drastically different among species for bagged and open fruits, and all fruit species persisted longer when protected against seed dispersers. We found that those fruits that are quickly removed by vertebrates are nutrient-rich, but although the attack rate of pathogens is also high, these fruits have low contents of quantitative defenses such as tannins and phenols. Thus, we suggest that the fruit removal rate by seed dispersers is the primary factor selecting the levels of fruit defense. Likewise, nutrient-poor fruits have low removal of seed dispersers and low probability of attack by pathogens. These species retain ripe fruits in an intact condition for a prolonged period because they are highly defended by secondary compounds, which reduce overall attractiveness. However, this strategy might be advantageous for plants that depend on rare or unreliable dispersers.

Keywords Fruit pathogens · Fruit removal · Secondary compounds · Plant–animal interactions

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Introduction

To overcome the constraints imposed by immobility, many plants produce fruits and flowers to recruit animal vectors for reproduction and dispersal. An often overlooked disadvantage of this strategy is that the nutritional rewards of fruits and flowers also attract antagonists that consume those rewards without providing reproductive benefits to the plant. The evolution of fruit and flower traits is thus assumed to respond to the sum of selective pressures exerted by mutualists and antagonists (Cipollini and Levey 1997a; Whitney and Stanton 2004). Teasing apart the relative influence of each consumer type on plant traits is hindered by the complexity and diffuse nature of their interactions with plants (Irwin et al. 2003; Whitney and Stanton 2004).

The interactions between fruiting plants, mutualists and antagonists are usually mediated by secondary metabolites. These compounds have complex functions in ripe fruits because fruit consumption can be either beneficial or detrimental to plants, depending on whether the consumer disperses or destroys the seeds (Levey et al. 2006). The defense trade-off hypothesis is the most influential framework to explain the relative contents of nutrients and secondary compounds in fruit pulp (Cipollini and Levey 1997a, b). This hypothesis is based on the assumption that secondary compounds in ripe fruits represent a trade-off with respect to defense against damaging agents and palatability for dispersers (Herrera 1982; Cipollini and Levey 1997b). There are two alternative models of this hypothesis leading to contrasting predictions on the relative amount of nutrients and secondary compounds in fruits. The removal rate model suggests an inverse relationship between nutrients and secondary compounds because nutrient-rich fruits should be quickly removed and thus should require few chemical defenses. In contrast, the nutrient/toxin titration model posits a positive correlation because nutrient-rich fruits should be profitable enough to allow for the retention of higher levels of defense (Cipollini and Levey 1997b).

To date, only Schaefer et al. (2003) has tested the two models of fruit biochemistry interspecifically, in a study comparing 33 plant species. This study supported the removal rate model concluding that nutrients stimulated fruit removal by seed dispersers while secondary compounds deterred it. However, fruit consumption by antagonists was not measured in this study and other similar studies on limited numbers of fruit species (Cipollini and Stiles 1993; Cipollini and Levey 1997a). Even though, Tang et al. (2005) also excluded seed dispersers from fruits and explained the variation in persistence time of these fruits with variation in defense against pathogens, they did not measure the amount of secondary compounds, and therefore the predictions of the models on fruit biochemistry could not be directly tested.

To test the alternative models on fruit composition we performed a controlled experiment on the relative fruit removal rates of mutualists and pathogens. To measure fruit removal by pathogens we excluded seed dispersers from fruits and compared persistence time of these fruits to control fruits that were freely accessible on the plant. By excluding seed dispersers we also excluded vertebrate seed predators. However, we minimized this effect by selecting species that are very rarely consumed by vertebrate seed predators such as parrots and pigeons. In a broad study of fruit-eating birds in the Atlantic forest, seed predators were only responsible for 2.3% of the total visits of frugivorous birds (Pizo 2004). The mesh size of the nets that we used to cover fruits did not exclude fungi, bacteria, and small insects. These organisms are the most ubiquitous seed predators (Cipollini and Levey 1997c), and exert indirect negative effects on plant fitness, because fruit rot deters frugivores and direct negative effects through seed destruction (Cipollini and Levey 1997c). However, the importance of these pathogens has often been ignored in theories of fruit–disperser interactions (but see Janzen 1977; Herrera 1982; Cipollini and Stiles 1992a; Cipollini and Levey 1997c). We therefore tested whether fruit consumption explains fruit removal rates of vertebrates and attack rate of invertebrates and pathogens. Based on the defense trade-off hypothesis we predicted that fruits rich in secondary compounds should have higher persistence time (i.e., lower attack rates by insects, fungi, and bacteria) and lower fruit removal by vertebrates than fruits with low contents of secondary compounds. If the removal rate model explains fruit pulp biochemistry, we predicted that nutrient-rich fruits should have lower persistence time because they are quickly removed by vertebrate seed dispersers. Conversely, fruits that are removed slowly should be highly defended and consequently persist for longer periods of time. If the alternative nutrient/toxin titration model explains fruit pulp contents, we expected that all fruits are under selection pressure for high fruit defense, but only nutrient-rich fruits are able to retain high levels of secondary metabolites.

We tested these predictions of the alternative models in a sub-sample of a tropical plant community. We evaluated fruit morphology, nutrient contents, and secondary compounds of these species and compared removal rates of vertebrate fruit consumers and pathogens. The specific goals of this study were: (1) to test whether nutrient rich fruits are more attractive to both frugivores and pathogens; (2) to evaluate if strongly protected fruits persist longer when seed dispersers are excluded, as suggested by the defense trade-off hypothesis and (3) to evaluate which alternative model, the removal rate or toxin titration, better explain the pulp content of ripe fruits.

Materials and methods

Study site

Fieldwork was carried out on Ilha do Cardoso, a land-bridge subtropical island, in São Paulo state, southeastern Brazil (25° 05′ S; 47° 53′ W). The vegetation of the island is composed exclusively of Atlantic rain forest represented by five different types: mangroves, dune vegetation, restinga forest, lowland tropical rainforest, and highland tropical rainforest (Noffs and Baptista Noffs 1982).

We conducted the experiments in restinga forest (sandy forest) and in lowland tropical rainforest. Restinga is an ecosystem of the Atlantic rainforest biome that belongs to the group of pioneer formations with marine influence and

that is distributed over a quaternary coastal plain (Ab'Saber 1955). The vegetation is characterized by low and medium canopy height (3–15 m) composed of trees with branched trunks (Sugiyama 1998; Couto and Cordeiro 2005). The lowland tropical Atlantic rain forest is characterized by an understory level and a relatively continuous and dense canopy that can reach 20 m with large amounts of epiphytes and vines.

The climate is generally warm and wet throughout the year but may be divided into two seasons: a cold and drier period from April to August when temperature may drop to nearly 13°C and rainfall is ca. 500 mm, and a warm and rainier period from September to March when temperature may reach 32°C and rainfall 1,800 mm (Oliveira-Filho and Fontes 2000).

Study species

This study examined fruit removal and characteristics of 14 plant species, all originating from different families. We used a conservative approach selecting species from different families in order to minimize phylogenetic effects, because most differentiation of nutritional fruit traits occurs at the level of genera (Jordano 1995). We selected common shrubs ranging from 1 to 4 m, and two species of vines (*Smilax brasiliensis and Cissampelos pareira*). We selected species for which we had detailed information on the suite of seed dispersers (Francisco and Galetti 2001, 2002; Pizo et al. 2002; Manhães 2003; Schrerer et al. 2007, as well as personal observations). All fruits were ornithocoric ranging from 3.8 to 14.4 mm in size. The main seed dispersing vertebrates are small birds, such as tanagers, thrushes, and flycatchers (see Table 1). Parrots and pigeons, which are important seed predators at Ilha do Cardoso, do not forage in the forest understory and we did not find any evidence of small mammals consuming the fruits of the studied species. Owing to detailed information on the range of frugivores that regularly consume these fruits, we considered insects and pathogens, such as fungi and bacteria, the main seed predators of the species that we evaluated.

Fruit removal

We selected 8 to 10 individuals of each species to evaluated fruit removal. The experiments took place in late summer (February and March) of 2005 and 2006, but observations extended for many months for some species. In each individual plant we covered four branches with nets and marked four other branches but left fruits open to seed dispersers (see Tsahar et al. 2002). The number of fruits present on each branch and those that had fallen into the nets in the bagged treatment was counted every 5 days, except for species with a long persistence time, like the palm *Geonoma schottiana* (Arecaceae), which we checked every fortnight. The number of fallen fruits was used as a surrogate for pathogen attack, because alterations of fruit tissue via fungal degradation can decrease persistence time by hastening abscission (Cipollini and Stiles 1992b). Even though we cannot distinguish between natural abscission and fruit rot, a large proportion of fruits fallen into the nets showed signs of attack by pathogens (small wounds, color changes). Thus, we assumed that fruit rot was the primary reason for abscission. We always set up the experiment before the fruits became ripe and began the experiment at maturation. Individual fruits in the bagged treatment were classified as "fallen", if they were found in the net, or "persisted", if they were still attached to the plant. Fruits of the open treatment were classified as "removed", if they disappeared from the plant, or "not removed", if they persisted on the plant. In each treatment we calculated the mean persistence time of fruits for each individual and used it for further analysis. To calculate removal efficiency we subtracted the mean number of fruits fallen into the nets from the mean number of missing fruits on the exposed branches and computed removal efficiency as the percentage of the total fruit crop from each tree (for details, see Tsahar et al. 2002).

We counted the number of fruits for all individuals. When crop size was small, all fruits were counted, otherwise crop size was estimated dividing the plant into four equal sections and counting all fruits present in one part and extrapolating it to the other ones. We also measured plant height and the distance from other fruiting trees of the same species (d1) and of fruiting trees of different species (d2) in an area of 15 m radius to delineate a patch of fruit resources, as it might be perceived by birds (Saracco et al. 2004).

Morphological traits

We collected 30 fruits of at least three different individuals to study fruit morphology. For each fruit we recorded: (1) length and diameter of the fruit and seeds; (2) fruit dry and fresh mass; (3) seed mass; (4) number of seeds per fruit. From these measurements we calculated two ratios: (1) relative yield of a fresh fruit (dry mass of the pulp/fresh mass of whole fruit); (2) pulp to seed ratio (dry mass of pulp/mass of the seeds).

Chemical analyses

For the chemical analyses, fruit pulp from at least three individuals of each species was frozen until the moment of analysis. Proteins were determined according to the Kjeldahl method, in which total protein is calculated by multiplying total nitrogen by 6.25 (Jeffery et al. 1989). Lipid contents were determined according to the macro-gravimetric method (Bligh and Dyer 1959) and the contents of

Table 1 Bird species that consume the fruits of the studied species

Frugivores	St	Gs	Mr	Gb	Ea	Op	Or	Cp	Ru	Mm	Go	Pn	Sb	Tb
Characidae														
Penelope superciliaris		2						2						
Mimidae														
Mimus saturninus						4,8								
Psittacidae														
Pyrrhura frontalis	7													
Ramphastidae														
Selenidera maculirostris		7												
Thraupidae														
Nemosia pileata									3*					
Ramphocelus bresilius	2		2	2						9			2	
Schistochlamys ruficapillus		5			4			5	6*					
Stephanophorus diadematus	7													
Tangara cayana							2		3*					
Thraupis sayaca	2		2	2	2	4,8	2		3*,8				2	
Thraupis ornata	7													
Turdidae														
Turdus albicollis						4,8			8		8	9		
Turdus amaurochalinus					8*	4,8		2	8		2			1
Turdus leucomelas						4			3*					
Turdus rufiventris						4,8			8		2			1
Tyrannidae														
Elaenia flavogaster						4,8			8					
Elaenia obscura										9				
Elaenia sp.						4,8			8				2	1
Empidonomus varius						4								
Megarhynchus pitangua									3*					
Myiarchus tyrannulus						4				9				1
Myiodynastes maculatus				2		4							2	
Myiozetetes similis	2					4							2	
Pitangus sulphuratus	2					4,8								
Tyrannus melancholicus						4			3*					
Tyrannus savana	2			2		4							2	1
Picidae														
Colaptes melanochorus									3*					
Pipridae														
Antilophia galeata									3*					
Carpornis cucullatus										6*	6		6*	
Thamnophilidae														
Thamnophilus caerulescens	7													
Vireonidae														
Vireo olivaceus									3*					

St *Schinus terebinthifolius*; Gs *Geonoma schottiana*; Mr *Maytenus robusta*; Gb *Gaylussacia brasiliensis*; Ea *Erythroxylum amplifolium*; Op *Ocotea pulchella*; Or *Ossaea retropila*; Cp *Cissampelos pareira*; Ru *Rapanea umbellata*; Mm *Myrcia multiflora*; Go *Guapira opposita*; Pn *Psychotria nuda*; Sb *Smilax brasiliensis*; Tb *Ternstroemia brasiliensis*

1 E. Cazetta, unpublished data (15 h of observation); *2* E. Cazetta, personal observation; *3* Francisco and Galetti (2001) (60 h of observation); *4* Francisco and Galetti (2002) (72 h of observation); *5* Manhães (2003); *6* Pizo et al. (2002); *7* M.A. Pizo, personal communication; *8* Schrerer et al. (2007); *9* Staggemeir and Guaraldo, personal communication

* Observations for species of the same genus

glucose, fructose, and sucrose by gas chromatography–mass spectrometer (modified from Pooter and Villar 1997). We used the mean gross energy equivalents of protein (17.2 KJ/g), lipid (38.9 KJ/g), and sugars (17.2 KJ/g, Karlson 1972) to determine the energetic value of dried fruit pulp for each species.

To evaluate plant defenses, we determined the contents of phenols and condensed tannins in fruits. We used these compounds for two reasons. First, they are the classical example of quantitative defenses, which deter herbivores (Janzen 1974; Harborne 1979, 1980; Laks 1989). There are numerous examples of their general antifungal, antibacterial, and antiherbivore activities (Davidson and Juneja 1990; Scalbert 1991; Cipollini and Stiles 1993; Panizzi et al. 2002), Moreover, they also deter vertebrate herbivores and frugivores (Provenza et al. 1990; Clark et al. 1991; Guglielmo et al. 1996; Schaefer et al. 2003). Second, phenols and tannins are among the most widespread secondary compounds in ripe fruits (van Buren 1970; Herrera 1982; Cipollini and Stiles 1992b; Foley et al. 1995). In a broad interspecific comparison such as our where species originate from different plant families, it is crucial to use such widespread compounds that occur in fleshy fruits. A potential caveat is that we use the total contents of phenols and tannins as a proxy of biological activity, while some compounds might not have effects on fruit consumers. As this problem reduces the likelihood of finding a consistent pattern between the contents of secondary compounds and fruit consumption of vertebrates and pathogens, we consider our approach conservative. We extracted these compounds by the Price and Butler (1977) method in butanol and methanol extracts (see Schaefer et al. 2003). The contents of these compounds were analyzed with photometric measurements. For each fruit sample, we ran three replicates and used the mean of the three values for statistical analyses.

Data analysis

In a seminal study on fruit characteristics in 910 species, Jordano (1995) found that chemical and morphological fruit traits were decoupled. We therefore used two principal component analyses (PCA) with Varimax rotation to evaluate major independent trends in the variation of morphological and chemical fruit traits, similar to previous studies (Schaefer et al. 2003). Separate PCA were used because combining suites of traits having low correlation between each other results in PC analyses that do not capture significant variation in these traits. We included energy in these analyses, even though it is a compound variable, because birds might forage on total energy contents rather than separately on the contents of lipids, proteins and sugar.

The mean persistence time for all species of bagged and open was compared using Wilcoxon signed rank test. The mean persistence time of bagged fruits was compared with the mean persistence time of open fruits across all species with a paired *t* test. The rate at which ripe fruits disappeared was estimated using Kaplan–Meier survival estimates (Willson and Whelan 1993; McCarty et al. 2002). Survival analysis is used when the response variable of interest is a time period until an event occurs. This type of analysis allows the inclusion of events that did not occur, because of the end of an experiment or individual loss (i.e., right-censored data). We calculated one rate for bagged fruits and one rate for open fruits.

We performed different multiple regressions with the two survival rates as response variables. We obtained the same results with forward and backward stepwise procedures. All survival rates were log transformed prior to analysis. We used factor scores of all the chemical principal components and the first morphological principal components as independent variables. We excluded the second and third morphological PCs because we were particularly interested in the effects of chemical variables based on our study questions. Moreover, previous studies reported that chemical characters are more important than morphological ones (Schaefer et al. 2003; Tang et al. 2005) and the first morphological PC accounts for most of morphological variance allowing us to correct for fruit morphology.

Results

Fruit traits

The PCA on morphological traits revealed that the first three components accounted for 87.45% of total variance. Fruit size (diameter and length) was correlated with fruit mass (Table 2). Variation in these traits represented the first principal component (PCM1) explaining 41.75% of the overall variation. The second principal component (PCM 2) is associated with seed size and mass. Fruits with low pulp to seed ratio and high relative yield show high values on the third principal component (PCM3) (Table 3).

The PCA for nutritional traits identified three components that accounted for 84% of total variance. Energy was positively correlated with lipids and negatively correlated with water (Table 4). Variation in these traits represented the first principal component (PCQ1), explaining 39.32% of the variation. Fruits with low contents of phenols and condensed tannins scored high on the second principal component (PCQ2), explaining 30.52% of the overall variation. Fruits with low protein contents scored high on the third principal component (PCQ3) (Table 5).

The first chemical principal component was correlated with the second and third morphological factors ($r_s = 0.54$, $P = 0.04$; $r_s = 0.68$, $P = 0.007$; respectively). Hence, fruits

Table 2 Spearman correlation of morphological fruit traits

	RY	Pulp/seed	No. seeds	1 seed mass	Seed length	Seed diam.	Fruit mass	Fruit lenght
Fruit diameter	−0.35	0.30	0.58*	0.39	−0.07	0.10	0.89*	0.59*
Fruit length	0.32	−0.21	0.28	0.64*	0.34	0.16	0.49**	
Fruit mass	−0.21	0.42	0.52	0.22	−0.20	−0.09		
Seed diameter	−0.13	−0.05	−0.50	0.73**	−0.30			
Seed length	0.25	−0.22	−0.30	0.56*				
1 seed mass	−0.11	0.01	−0.28					
No. seeds	−0.16	0.22						
Pulp/seed	−0.57*							

* $P < 0.01$, ** $P < 0.001$

Table 3 Principal component analysis of morphological traits (only factor loadings >0.5 are shown)

Morphological traits	PCM1	PCM2	PCM3
Fruit diameter	**0.95**		
Fruit length	**0.92**		
Fruit mass	**0.89**		
1 seed mass	0.51	**0.82**	
Seed number		**−0.66**	0.54
Seed diameter		**0.98**	
Pulp/seed	0.94		**−0.81**
RY	−0.58		**0.74**
Eigenvalue	3.34	2.28	1.37
% variance explained	41.75	28.51	17.18
Cumulative variance	41.75	70.26	87.45

Major relationships (factor loadings > 0.6) are typed in boldface

Table 4 Spearman correlation of nutritional fruit traits

	Tannins	Phenol	Water	Energy	Sugar	Protein
Lipids	−0.03	0.12	−0.46	0.93**	−0.3	−0.1
Proteins	0.14	0.44	−0.22	0.12	−0.1	
Sugar	0.10	−0.19	0.35	−0.30		
Energy	0.05	0.16	−0.56*			
Water	0.18	0.16				
Phenol	0.45					

* $P < 0.01$, ** $P < 0.001$

with high lipids and energy contents had higher seed mass, seed diameter and relative yield and lower pulp/seed ratio. The second chemical principal component was negatively correlated with the second morphological factor ($r_s = -0.76$, $P = 0.001$). Fruits with high phenols and tannins contents had higher seed mass and seed diameter.

Fruit fates

The mean fruit removal efficiency was 57.29% (range 26.6–83.5%). Fruit removal efficiency of four species was around 80% and *Ossaea retropila* (Melastomataceae) and *Myrcia multiflora* (Myrtaceae) had the lowest removal efficiency (26.5 and 32.9%, respectively) (Table 5). There was no correlation between removal efficiency and crop size ($r_s = 0.17$, $P = 0.09$), distance to other fruiting trees (D1: $r_s = 0.06$, $P = 0.57$; D2: $r_s = 0.03$, $P = 0.86$), and plant height ($r_s = -0.08$, $P = 0.40$). We likewise found no influence of these variables if they were included as co-variates in General Linear Models (GLM) with fruit removal of bagged and unprotected fruits as dependent variable.

Table 5 Principal component analysis of nutritional traits (only factor loadings >0.5 are shown)

Chemical traits	PCQ1	PCQ2	PCQ3
Lipids	**0.95**		
Proteins			**−0.97**
Sugar	−0.51		
Energy	**0.95**		
Water	**−0.80**		
Phenols		**−0.78**	−0.52
Tannins		**−0.94**	
Eigenvalue	2.75	2.13	0.99
% variance explained	39.32	30.52	14.17
Cumulative variance	39.32	68.85	84.03

Major relationships (factor loadings > 0.6) are typed in boldface

The mean persistence time of bagged and open fruits across all species was different ($t = 11.14$, $P < 0.0001$). Fruits protected against frugivores persist longer than fruits open to them (Fig. 1; Table 6). We also found interspecific differences in the exposure time of ripe fruits in both treatments: when seed dispersers were excluded (Wilcoxon, $P < 0.0001$) or included (Wilcoxon, $P < 0.0001$).

Influence of fruit traits on persistence and removal

Fruit characteristics influenced persistence time of bagged and open fruits. Fruits with high lipid and energy contents persist for a short period of time, whereas fruits with high phenol and tannin contents persist for long periods in both treatments. Fruit size influences the persistence time only of

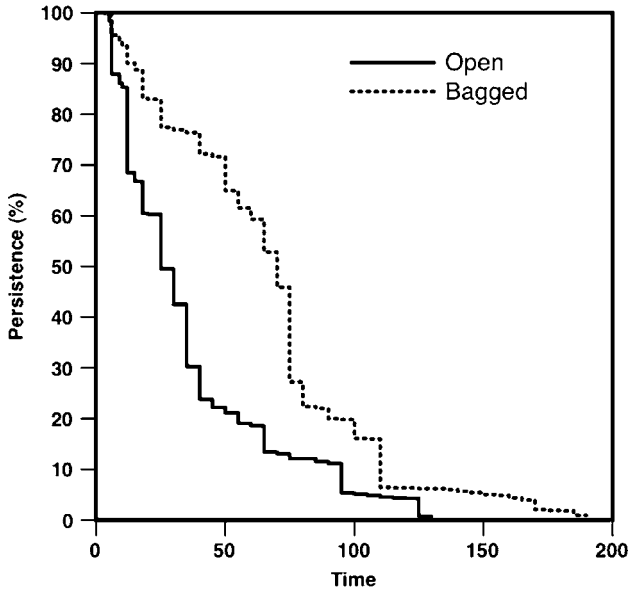

Fig. 1 Fruit persistence in the open and bagged treatment of all species evaluated, based on Kaplan–Meier survival estimates

bagged fruits so that larger fruits persisted for a shorter period than small fruits when vertebrates were excluded (Table 7).

Discussion

This study evaluated for the first time the influence of nutrients and secondary compounds on fruit consumption by vertebrate seed dispersers and pathogens. We found that fruit species that are quickly removed by both, vertebrate seed dispersers and pathogens, are nutrient-rich and contain lower amounts of phenols and condensed tannins. In contrast, fruit species that persisted longer, both when vertebrate consumers were excluded or when they had access to the fruits, are nutrient-poor and contain higher amounts of these secondary compounds. Thus, our results corroborate the removal rate model of the defense-trade off hypothesis.

Our results showed that fruits with high lipid and energy contents are quickly removed in all treatments. Frugivores may forage for fruits that best satisfy their nutritional demands, as stated by the nutrient content hypothesis (Manasse and Howe 1983; Denslow 1987; Levey 1987). However, pathogens also prefer nutrient-rich fruits and thus select against them. Our result suggests that we can predict how fruiting plants, seed dispersers, and pathogens interact simultaneously with the other players in the evolutionary triad (Tsahar et al. 2002; Tewksbury 2002). We suggest that according to the relative speed of fruit removal, plant species are located on different positions along the continuum of optimal defenses against antagonists and optimal attraction to seed dispersers.

The defense trade-off hypothesis builds on the assumption that secondary compounds mediate fruit persistence by their antimicrobial and antifungal effects (Jones and Wheelwright 1987; Cipollini and Stiles 1992b). We corroborate this notion because fruits with high phenols and tannins persisted for long time periods when vertebrates were excluded. We admit that natural abscission may have biased our estimates of the attack rate of antagonists in our experiment. This might be true particularly for bigger fruits, which persisted for shorter periods when vertebrates were excluded. However, the overall negative relationship between secondary compounds and fruit persistence time is unlikely to be explained primarily by natural fruit abscission. We therefore believe that the effects of natural abscission do not change the main result of this study.

Table 6 Mean crop size, removal efficiency, and the time that fruits remain available (persistence in days—mean ± S.E.) for bagged (B) and open fruits (O)

Species	Crop size	Removal efficiency (%)	B	O
Cissampelos pareira	740	45.45	68.82 ± 0.3	59.66 ± 0.57
Ossaea retropila	211	26.56	39.64 ± 0.13	33.19 ± 0.27
Erythroxylum amplifolium	18	50	11.24 ± 0.37	10.05 ± 0.61
Gaylussacia brasiliensis	107	56.6	61.75 ± 0.88	51.65 ± 0.70
Geonoma schottiana	300	48.5	150	119.26 ± 0.12
Guapira opposita	250	75	26.73 ± 0.07	16.79 ± 0.22
Maytenus robusta	977	32.91	69.42 ± 0.93	62.62 ± 0.69
Myrcia multiflora	102	80.7	57.51 ± 0.55	42.35 ± 0.41
Ocotea pulchella	148	50	33.18 ± 0.13	29.93 ± 0.004
Psychotria nuda	28	79.26	26.85 ± 0.98	18.96 ± 0.46
Rapanea umbellata	3733	83.47	90	78.74 ± 0.23
Schinus terebinthifolius	8373	50	55	37.49 ± 0.09
Smilax brasiliensis	458	44.6	172.72 ± 0.32	126.85 ± 0.57
Ternstroemia brasiliensis	212	79	14.96 ± 0.02	11.93 ± 0.03

Table 7 Multiple regression for the persistence rate of bagged and open fruits. Only significant independent variables are shown

Dependent variable	Source	Regression coefficient	SE	*T*	*P*	Standard regression coefficient	R^2 (%)	*P*
Bagged	PCM1	−0.1	0.051	−1.99	0.048	1.04	60	<0.0001
	PCQ1	−0.44	0.051	−8.82	<0.0001	20.42		
	PCQ2	−0.43	−0.43	−8.98	<0.0001	21.16		
Open	PCQ1	−0.37	0.055	−6.73	<0.0001	16.02	52	<0.0001
	PCQ2	−0.49	0.056	−8.87	<0.0001	27.81		

Avian frugivores also avoided fruits rich in phenols and tannins suggesting that the increase of fruit persistence comes at a cost owing to a concomitant decrease in the palatability of fruits to seed dispersers (Foley et al. 1995; Schaefer et al. 2003). While many other classes of secondary compounds contribute to plant defense, e.g., glycoalkaloids (Cipollini and Levey 1997a; Levey and Cipollini 1997; Wahaj et al. 1998), our results demonstrate that phenols and tannins predict both, fruit avoidance of vertebrates and persistence time of fruits in the absence of fruit removal by vertebrates. This pattern fits the conjecture that phenols and tannins constitute quantitative plant defense (van Buren 1970; Harborne 1979; Herrera 1982). The presence of phenols might further be correlated with that of other secondary compounds with strong defensive activity (e.g., compounds produced by the phenyl pathway Fineblum and Rausher 1997).

We found an inverse relationship between levels of nutrients and phenols and condensed tannins; plant species that were quickly removed by dispersers and pathogens contained more nutritional reward and lower amounts of secondary compounds. Although pathogens also preferentially attack fruits rich in nutrients, these fruits retain low levels of defense. This paradox is explicable because the removal rate of seed dispersers is higher than that by pathogens. Consequently, the most rewarding fruit species are attractive enough to seed dispersers to offset the detrimental effects of pathogens. The removal rate model thus explains the pulp composition of the plant species that we studied. Previous studies also supported the removal rate model, concluding that nutrients stimulated fruit removal by seed dispersers while secondary compounds deterred them (Cipollini and Stiles 1993; Cipollini and Levey 1997a; Schaefer et al. 2003).

While nutrient-rich fruits are quickly consumed by mutualists and thus are poorly defended, nutrient-poor fruits are neither preferred by vertebrate fruit consumers nor attacked quickly by pathogens. Because the probability of a ripe fruit being attacked by antagonists increases linearly with exposure time (Thompson and Willson 1978), long persisting fruits are highly defended by secondary compounds. We suggest that—over evolutionary time—these species improved their competitive ability by higher investment in fruit defense. This strategy might be particularly important for plant species that depend on rare or unreliable seed-dispersers (Cipollini and Stiles 1992c; Tang et al. 2005). For dispersers that consume these fruits sporadically, the quantities of deterrent substances will not exceed critical levels. Deterrent compounds might thus not strongly affect the intrinsically low individual consumption rates inherent to this kind of fruits (Herrera 1982).

Although the nutritional value of the fruits and the digestive ability of birds play a major role in fruit choice (Martinez del Rio and Restrepo 1993), many studies on seed dispersers have demonstrated the importance of characteristics such as fruit abundance, crop size, neighborhood effect, fruit size, color, among others (Sargent 1990; Murphy 1994; Saracco et al. 2005, etc.). However, as far as we know, similar studies evaluating how these characteristics influence fruit consumption by pathogens have never been conducted. We evaluated some of these traits in the present study but found no influence on fruit removal by vertebrates and pathogens. Plant characteristics such as height and crop size and neighborhood effects also did not influence fruit removal by seed dispersers in our study.

In conclusion, we demonstrate that chemical characteristics primarily influence preferences and removal rate by a broad range of frugivores. We found that nutrient-rich fruits are simultaneously attractive to both, pathogens and vertebrates. In spite of the high attack rate of pathogens, these fruits are poorly defended by secondary compounds suggesting that the removal rate of seed dispersers is the primary factor controlling the level of fruit defense. Selection for defense is high for fruits with low nutrient quality that are less preferred and consequently removed slowly. Because these species are highly defended, they retain ripe fruits in an intact condition for a prolonged period, which in turn reduces attractiveness but might be advantageous for plants that depend on rare or unreliable dispersers.

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Appendix I - Morphological and nutritional fruit traits of the 14 plant species evaluated on Ilha do Cardoso.

	Species	Fruit diam.	Fruit length	Fruit mass	Seed diam.	Seed length	1 seed mass	N. seeds	Pulp/ seed	RY	Lipids	Protein	Sugar	Energy	Water	Phenol	Tannin
Anacardiaceae	*Schinus terebinthifolius*	5.44	4.65	0.01	3.63	21.5	0.02	1	0.6	0.34	5.47	6.86	0.87	3.53	65.91	2.3	0.02
Arecaceae	*Genoma schottiana*	9.56	9.45	0.3	6.68	7.12	0.2	1	1.5	0.21	5.43	9.35	0.03	3.8	79.07	4.5	0.17
Celastraceae	*Maytenus robusta*	8.71	11.25	0.11	5.7	7.58	0.13	1.47	0.9	0.24	1.79	2.63	0.18	1.2	76.38	0.61	0.08
Ericaceae	*Gaylussacia brasiliensis*	7.44	6.7	0.22	1.79	2.68	0.02	9.2	13.8	0.13	5.48	2.52	3.01	3.14	87.48	1	0.03
Erythroxylaceae	*Erythroxylum amplifolium*	5.72	7.48	0.11	3.67	6.95	0.04	1	2.55	0.69	26.87	4.59	0.07	11.39	31.29	0.68	0.02
Lauraceae	*Ocotea pulchella*	5.75	8.17	0.08	4.6	7.19	0.08	1	0.98	0.34	60.57	5.19	0.16	24.76	65.65	1.93	0.05
Melastomataceae	*Ossaea retropila*	8.77	8.22	0.31	0.1	0.1	0	539.8	0.31	0.47	2.97	5.98	1.74	2.54	77.14	0.81	0.04
Menispermaceae	*Cissampelos pareira*	7.31	7.3	0.17	4.93	5.61	0.04	1	4.57	0.44	37.3	10.69	0.06	16.57	56.02	4.01	0.12
Myrsinaceae	*Rapanea umbellata*	6.31	5.61	0.1	3.68	4.22	0.03	1	3.54	0.14	7.98	2.86	0.1	3.67	86.18	2.88	0.27
Myrtaceae	*Myrcia multiflora*	5.91	5.22	0.1	3.71	3.15	0.02	1.1	5	0.2	11.65	5.29	2.98	6.05	80	0.67	0.05
Nyctaginaceae	*Guapira opposita*	6.53	6.95	0.16	3.84	6.08	0.05	1	2.97	0.16	2.27	8.83	0.14	2.49	84	1.12	0.02
Rubiaceae	*Psychotria nuda*	14.45	13.59	1.14	3.11	7.86	0.1	1.9	11.97	0.2	2.75	7.3	2.06	2.75	79.84	0.64	0.04
Smilacaceae	*Smilax brasiliensis*	7.41	7.33	0.15	5.29	5.26	0.1	1	1.5	0.13	3.78	4.47	0.02	2.29	85.84	0.88	0.02
Theaceae	*Ternstroemia brasiliensis*	3.8	7.33	0.01	3.54	5.83	0.03	1	0.3	0.48	83.33	3.49	0	33.37	52.34	0.52	0.01

CONCLUSÕES GERAIS

Neste trabalho demonstramos que as características morfológicas e químicas dos frutos em geral apresentam sinal filogenético e estão pouco associadas entre si. Isso indica que a seleção natural não favorece combinações particulares de características, como o esperado para as síndromes de dispersão de sementes. Ao contrário, as cores dos frutos, quando avaliadas com um espectrômetro e não categorizadas segundo a visão humana, como ocorre na maioria dos estudos de dispersão, não apresentam sinal filogenético e podem ser consideradas como adaptações aos animais frugívoros, como sugerido pelos primeiros naturalistas. Além disso, quanto maior a saturação dos frutos, maior a quantidade de lipídeos e energia, o que revela que frutos mais saturados são um sinal verdadeiro para os animais frugívoros.

Considerando a preferência das aves frugívoras e avaliando as cores dos frutos como um sinal visual para aumentar a detectabilidade, nós notamos que as aves frugívoras encontram frutos artificiais com maior contraste cromático, enquanto o contraste acromático parece não influenciar na detecção. Corroborando esse fato, observamos que os frutos naturais presentes na floresta de planície, onde a intensidade luminosa é menor, apresentam maiores contrastes cromáticos do que os frutos da restinga, em que a intensidade luminosa é muito maior. Portanto, nós sugerimos que um aumento no contraste cromático pode ser vantajoso para as plantas principalmente em ambientes com pouca ou variável intensidade luminosa.

As aves frugívoras também removeram mais rapidamente frutos ricos em energia e lipídeos e pobres em compostos secundários. Os frugívoros antagonistas, como fungos e bactérias, também preferem os mesmos tipos de frutos. Portanto, nós sugerimos que a relação entre atração e defesa em frutos carnosos é moldada pelas taxas

de remoção das aves frugívoras e não como pensado previamente pela concentração de compostos secundários. Desta maneira, frutos removidos mais rapidamente por dispersores legítimos são pobres em compostos secundários, enquanto frutos removidos lentamente são mais defendidos por esses compostos podendo permanecer por mais tempo na planta e desta forma atrair dispersores raros ou eventuais.

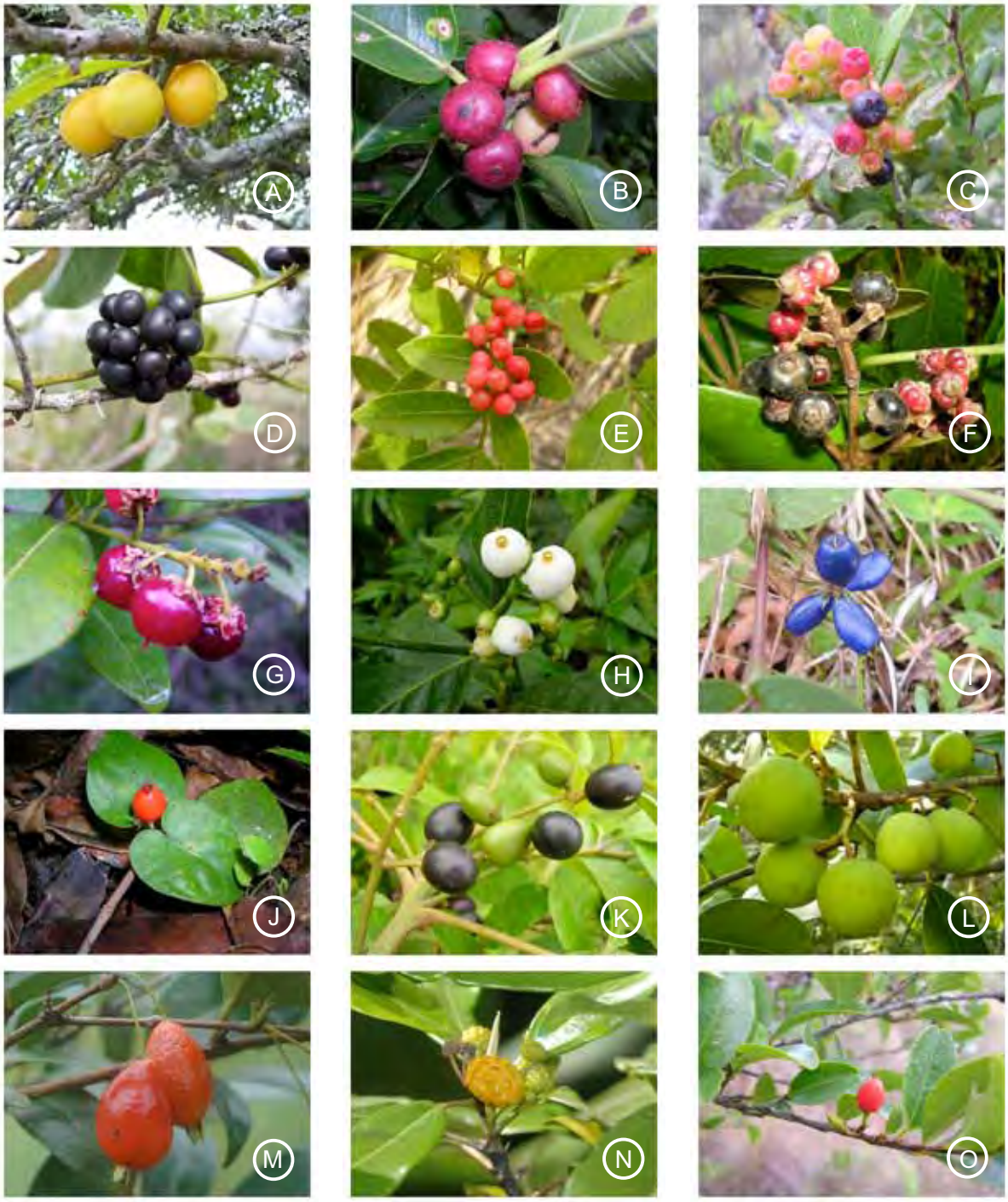
Embora as aves tenham detectado mais facilmente frutos com maior contraste cromático e consumido preferencialmente frutos ricos em lipídeos e energia e pobres em compostos secundários, isso não opera na evolução das características dos frutos, uma vez que para esse fato ocorrer é necessário que as aves selecionem entre indivíduos da mesma espécie e não entre espécies diferentes. Embora a seleção de determinadas características dos frutos pelas aves possa não acarretar na evolução destas características, como parece ser o caso das características químicas e morfológicas dos frutos, ainda assim a preferência das aves pode proporcionar recrutamento diferencial das espécies de plantas. Como a remoção dos frutos é o primeiro de uma série de processos seqüenciais que levam ao estabelecimento e recrutamento, a seleção de espécies com determinadas características pode aumentar o sucesso reprodutivo destas espécies.

Apêndice A

Exemplos de algumas espécies utilizadas neste estudo

A) *Cabralea canjerana* (Meliaceae), B) *Margaritaria nobilis* (Euphorbiaceae), C) *Ternstroemia brasiliensis* (Theaceae), D) *Maytenus robusta* (Celastraceae), E) *Virola bicuhyba* (Myristicaceae), F) *Xylopia langsdorfiana* (Annonaceae), G) *Heisteria silvianii* (Olacaceae), H) *Abarema brachystachya* (Leguminosae), I) *Doliocarpus glomeratus* (Dilleniaceae), J) *Psychotria leiocarpa* (Rubiaceae), K) *Guarea macrophylla* (Meliaceae), L) *Clusia criuva* (Clusiaceae), M) *Geonoma pauciflora* (Arecaceae), N) *Ossaea retropila* (Melastomataceae) e O) *Coccocypselum cordifolium* (Rubiaceae) .

Todas as fotos de autoria de E. Cazetta, exceto *F* de E.R. Castro



A) *Ximena americana* (Olacaceae), B) *Ficus organensis* (Moraceae), C) *Gaylussacia brasiliensis* (Ericaceae), D) *Smilax brasiliensis* (Smilacaceae), E) *Schinus terebinthifolius* (Anarcadiaceae), F) *Gomidesia fenzliana* (Myrtaceae), G) *Byrsonima ligustrifolia* (Malpighiaceae), H) *Rudgea heurckii* (Rubiaceae), I) *Coccocypselum lanceolatum* (Rubiaceae), J) *Geophila repens* (Rubiaceae), K) *Tapirira guianensis* (Anacardiaceae) e L) *Calophyllum brasiliense* (Clusiaceae), M) *Eugenia cuprea* (Myrtaceae), N) *Coussapoa microcarpa* (Urticaceae) e O) *Erythroxylum amplifolium* (Erythroxylaceae).

Todas as fotos de autoria de E. Cazetta, exceto *M* de E. R. Castro

A) *Vitex polygama* (Verbenaceae), B) *Psychotria mapourioides* (Rubiaceae), C) *Chioccoca alba* (Rubiaceae), D) *Ocotea pulchella* (Lauraceae), E) *Smilax quinquenervia* (Smilacaceae), F) *Rapanea umbellata* (Myrsinaceae), G) *Gatteria australis* (Annonaceae), H) *Cordia verbenaceae* (Boraginaceae) e I) *Rudgea villiflora* (Rubiaceae).

Todas as fotos de autoria de E. Cazetta, exceto *G* e *H* de E. R. Castro

Apêndice B

Características morfológicas, químicas e de coloração das espécies estudas na Ilha do Cardoso

Appendix I – Fruit characteristics of 105 plant species from Cardoso Island, Atlantic rainforest, Brazil.

Family	Species	Growth form	Habitat	Dispersor	Color
Anacardiaceae	*Schinus terebinthifolius*	Shrub	Restinga	*Bird*	Red
Anacardiaceae	*Tapirira guianensis*	Tree	Restinga	*Bird*	Red/Black
Annonaceae	*Guatteria australis*	Tree	Restinga	*Bird*	Black
Annonaceae	*Rollinia sericea*	Tree	Restinga	*Mammal*	Yellow
Annonaceae	*Xylopia langsdorfiana*	Tree	Restinga	*Bird*	Black/White
Aquifoliaceae	*Ilex amara*	Tree	Restinga	*Bird*	Black
Aquifoliaceae	*Ilex theazans*	Tree	Restinga	*Bird*	Black
Arecaceae	*Astrocaryum aculeatissimum*	Tree	R/L/H	*Mammal*	Brown
Arecaceae	*Attalea dubia*	Tree	Lowland	*Mammal*	Orange
Arecaceae	*Bactris setosa*	Shrub	Restinga	*Mammal*	Black
Arecaceae	*Euterpe edulis*	Tree	R/L/H	*Mixed*	Black
Arecaceae	*Geonoma gamiova*	Shrub	Highland	*Bird*	Black
Arecaceae	*Geonoma pauciflora*	Shrub	L/H	*Bird*	Black
Arecaceae	*Geonoma schottiana*	Shrub	Restinga	*Bird*	Black
Arecaceae	*Siagrus romanzoffiana*	Tree	Restinga	*Mixed*	Orange
Boraginaceae	*Cordia verbenacea*	H	Restinga	Bird	Red
Bromeliaceae	*Aechmea nudicaulis*	Bromelia	Restinga	*Bird*	Orange
Bromeliaceae	*Bromelia antiacantha*	Bromelia	Restinga	*Mammal*	Yellow
Burseraceae	*Protium heptaphyllum*	Tree	Restinga	*Bird*	White
Celastraceae	*Maytenus robusta*	Shrub	Restinga	*Bird*	White
Celastraceae	*Maytenus sp.*	Tree	Restinga	*Bird*	White
Chloranthaceae	*Hedyosmum brasiliense*	Tree	Restinga	*Bird*	White
Chrysobalanaceae	*Hirtella hebeclada*	Tree	Lowland	*Bird*	Black
Clusiaceae	*Calophyllum brasiliense*	Tree	Restinga	*Mixed*	Green
Clusiaceae	*Clusia criuva*	Tree	Restinga	*Bird*	Red
Clusiaceae	*Garcinia gardneriana*	Tree	Highland	*Mammal*	Yellow
Connaraceae	*Connarus sp.*	Tree	Highland	*Bird*	Black/Yellow
Dilleniaceae	*Doliocarpus glomeratus*	Vine	Restinga	*Bird*	White
Ericaceae	*Gaylussacia brasiliensis*	Shrub	Restinga	*Bird*	Red/Black
Erythroxylaceae	*Erythroxylum amplifolium*	Shrub	Restinga	*Bird*	Red
Euphorbiaceae	*Margaritaria nobilis*	Tree	Lowland	*Bird*	Blue
Lauraceae	*Cryptocarya moschata*	Tree	L/H	*Mixed*	Yellow
Lauraceae	*Ocotea pulchella*	Shrub	Restinga	*Bird*	Black
Loranthaceae	*Struthanthus concinnus*	Hemi	Restinga	*Bird*	Brown
Malpighiaceae	*Byrsonima ligustrifolia*	Tree	Restinga	*Mixed*	Black
Marcgraviaceae	*Norantea brasiliensis*	Vine	Restinga	*Bird*	Red

Family	Species	Growth form	Habitat	Dispersor	Color
Melastomataceae	*Miconia rigidiuscula*	Shrub	Restinga	Bird	Black
Melastomataceae	*Ossaea retropila*	Shrub	Restinga	Bird	Black
Meliaceae	*Cabralea canjerana*	Tree	Lowland	*Bird*	Red
Meliaceae	*Guarea macrophylla*	Tree	Lowland	*Bird*	Red
Menispermaceae	*Abuta selloana*	Tree	Lowland	*Mammal*	Yellow
Menispermaceae	*Cissampelos pareira*	Vine	Lowland	*Bird*	Red
Monimiaceae	*Mollinedia schottiana*	Tree	R/L	*Bird*	Black
Moraceae	*Ficus adhatadifolia*	Tree	Lowland	*Bird*	Green
Moraceae	*Ficus arpazusa*	Tree	Lowland	*Bird*	Green
Moraceae	*Ficus insipida*	Tree	Lowland	*Bird*	Green
Moraceae	*Ficus organensis*	Tree	Lowland	*Bird*	Red
Moraceae	*Ficus pulchella*	Tree	Lowland	*Bird*	Green
Moraceae	*Sorocea bonplandii*	Tree	Highland	*Mixed*	Black
Myristicaceae	*Virola bicuhyba*	Tree	L/H	*Bird*	Rosa
Myrsinaceae	*Cybianthus peruvianus*	Shrub	Restinga	Bird	Black
Myrsinaceae	*Rapanea umbellata*	Shrub	Restinga	*Bird*	Black
Myrsinaceae	*Rapanea venosa*	Tree	Restinga	*Bird*	Black
Myrtaceae	*Campomanesia guaviroba*	Tree	Lowland	*Mixed*	Yellow
Myrtaceae	*Campomanesia xanthocarpa*	Tree	Lowland	Mammal	Orange
Myrtaceae	*Eugenia cuprea*	Shrub	Lowland	*Bird*	Red
Myrtaceae	*Eugenia sp.*	Tree	Highland	*Mammal*	Orange
Myrtaceae	*Eugenia sulcata*	Shrub	Restinga	*Bird*	Black
Myrtaceae	*Eugenia umbelliflora*	Tree	Restinga	*Mixed*	Black/Red
Myrtaceae	*Gomidesia fenzliana*	Tree	Restinga	*Bird*	Red/Black
Myrtaceae	*Gomidesia flagelaris*	Tree	Highland	*Bird*	Black
Myrtaceae	*Gomidesia schaueriana*	Tree	Restinga	*Bird*	Yellow/Red
Myrtaceae	*Gomidesia spectabilis*	Tree	Lowland	*Bird*	Cinza
Myrtaceae	*Marlieria tomentosa*	Tree	R/L	*Mixed*	Black
Myrtaceae	*Myrcia bicarinata*	Tree	Restinga	*Bird*	Red/Black
Myrtaceae	*Myrcia insularis*	Tree	Lowland	*Mixed*	Black
Myrtaceae	*Myrcia multiflora*	Tree	Restinga	*Bird*	Black
Myrtaceae	*Myrcia rostrata*	Shrub	Restinga	*Bird*	Red/Black
Myrtaceae	*Pimenta pseudocaryiophyllus*	Tree	Restinga	*Bird*	Black
Myrtaceae	*Psidium cattleyanum*	Shrub	Restinga	*Mixed*	Yellow
Myrtaceae	*Siphoneugena guilfoyleiana*	Tree	Restinga	*Bird*	Red/Black
Nyctaginaceae	*Guapira opposita*	Tree	Restinga	*Bird*	Black

Family	Species	Growth form	Habitat	Dispersor	Color
Olacaceae	*Heisteria silvianii*	Tree	Highland	*Mixed*	White
Olacaceae	*Ximenia americana*	Shrub	Restinga	*Mammal*	Yellow
Quiinaceae	*Quiina glazovii*	Tree	L/H	*Mixed*	Orange
Rubiaceae	*Chiococca alba*	Shrub	Restinga	*Bird*	White
Rubiaceae	*Coccocypselum cordifolium*	Herb	Restinga	*Bird*	Blue
Rubiaceae	*Coccocypselum lanceolatum*	Herb	Restinga	*Bird*	Blue
Rubiaceae	*Geophila repens*	Herb	Lowland	*Bird*	Red
Rubiaceae	*Psychotria deflexa*	Shrub	Lowland	*Bird*	White
Rubiaceae	*Psychotria hoffmannseggiana*	Shrub	R/L	*Bird*	Black
Rubiaceae	*Psychotria kleinii*	Shrub	Lowland	*Bird*	Blue
Rubiaceae	*Psychotria leiocarpa*	Shrub	Lowland	*Bird*	Blue
Rubiaceae	*Psychotria mapourioides*	Tree	Lowland	*Bird*	Red/Orange/Yellow
Rubiaceae	*Psychotria nemorosa*	Shrub	Lowland	*Bird*	Lilás
Rubiaceae	*Psychotria nuda*	Shrub	Lowland	*Bird*	Blue
Rubiaceae	*Rudgea heurchkii*	Shrub	Lowland	*Bird*	White
Rubiaceae	*Rudgea villiflora*	Shrub	Restinga	*Bird*	Red
Sabiaceae	*Pouteria psammophila*	Tree	Lowland	Mammal	Green
Santalaceae	*Phoradendron hexastichum*	Hemi	Restinga	Bird	White
Sapindaceae	*Allophylus petiolulatus*	Tree	Lowland	*Bird*	Red
Sapindaceae	*Paullinia seminuda*	Vine	Lowland	*Bird*	White
Sapotaceae	*Manilkara subsericea*	Tree	Lowland	*Mammal*	Orange
Sapotaceae	*Pradosia lactescens*	Tree	Highland	Mammal	Yellow
Smilacaceae	*Smilax brasiliensis*	Vine	Restinga	*Bird*	Black
Smilacaceae	*Smilax quinquenervia*	Vine	Restinga	Bird	Orange
Solanaceae	*Cestrum sp.*	Shrub	Lowland	Mixed	Black
Solanaceae	*Solanum capsicoides*	Herb	Restinga	*Mammal*	Orange
Symplocaceae	*Symplocos laxiflora*	Tree	L/H	*Bird*	Blue
Theaceae	*Ternstroemia brasiliensis*	Shrub	Restinga	*Bird*	Red
Urticaceae	*Coussapoa microcarpa*	Tree	Lowland	*Bird*	Yellow
Urticaceae	*Pourouma guianensis*	Tree	Lowland	*Bird*	Black
Verbenaceae	*Vitex polygama*	Tree	Lowland	*Mixed*	Black
Vitaceae	*Cissus paullinifolia*	Vine	Restinga	Bird	Black
Vitaceae	*Cissus verticillata*	Vine	Restinga	Bird	Black

Appendix II – Morphological characteristics of 105 fruit species from Cardoso Island, Atlantic rainforest, Brazil.

Family	Species	Fruit diameter (mm)	Fruit length (mm)	Fruit fresh mass (g)	Pulp fresh mass (g)	Pulp dry mass (g)	Seed diameter (mm)	Seed length (mm)	Seed mass/ fruit (g)	Seed number	Pulp/seed	RY
Anacardiaceae	*Schinus terebinthifolius*	5.44	4.65	0.04	0.01	0.005	3.63	21.50	0.02	1.00	0.20	0.13
Anacardiaceae	*Tapirira guianensis*	10.94	12.09	0.88	0.59	0.13	8.15	10.44	0.30	1.00	0.43	0.15
Annonaceae	*Guatteria australis*	6.50	8.96	0.26	0.17	0.03	4.46	7.42	0.08	1.00	0.38	0.12
Annonaceae	*Rollinia sericea*	33.70	34.82	22.76	19.99	1.18	3.76	5.64	2.77	56.30	0.43	0.05
Annonaceae	*Xylopia langsdorfiana*	6.06	9.27	0.18	0.02	0.01	6.12	8.62	0.17	1.00	0.09	0.08
Aquifoliaceae	*Ilex amara*	6.19	5.04	0.12	0.10	0.02	2.24	3.52	0.02	3.63	0.90	0.17
Aquifoliaceae	*Ilex theazans*	7.21	7.45	0.23	0.18	0.05	2.70	4.75	0.05	3.70	1.03	0.22
Arecaceae	*Astrocaryum aculeatissimum*	31.21	42.50	19.70	5.87	3.40	27.95	38.86	13.84	1.00	0.25	0.17
Arecaceae	*Attalea dubia*	29.61	43.93	23.41	10.13	2.02	23.11	40.07	13.29	1.00	0.15	0.09
Arecaceae	*Bactris setosa*	19.14	19.29	4.06	2.16	0.86	15.89	12.03	1.90	1.00	0.45	0.21
Arecaceae	*Euterpe edulis*	13.63	12.67	1.52	0.46	0.16	12.05	11.30	1.06	1.00	0.15	0.10
Arecaceae	*Geonoma gamiova*	8.75	11.28	0.49	0.30	0.08	6.17	8.51	0.20	1.00	0.37	0.15
Arecaceae	*Geonoma pauciflora*	7.94	9.05	0.34	0.19	0.04	5.73	6.85	0.14	1.00	0.28	0.12
Arecaceae	*Geonoma schottiana*	9.56	9.45	0.50	0.30	0.06	6.68	7.12	0.20	1.00	0.31	0.13
Arecaceae	*Siagrus romanzoffiana*	21.07	21.16	6.30	3.50	1.50	15.32	19.97	2.77	1.00	0.54	0.24
Boraginaceae	*Cordia verbenacea*	6.95	6.44	0.17	0.16	0.02	2.91	4.07	0.02	1.00	1.00	0.12
Bromeliaceae	*Aechmea nudicaulis*	6.92	15.13	0.29	0.17	0.05	1.20	1.34	0.12	76.00	0.42	0.17
Bromeliaceae	*Bromelia antiacantha*	29.13	38.97	18.59	17.37	8.09	6.64	5.98	1.22	16.87	6.63	0.44
Burseraceae	*Protium heptaphyllum*	15.69	21.43	3.18	2.74	0.54	9.40	16.22	0.44	1.13	1.22	0.17
Celastraceae	*Maytenus robusta*	7.46	9.04	0.24	0.11	0.03	5.70	7.58	0.13	1.47	0.21	0.11
Celastraceae	*Maytenus sp.*	8.71	11.25	0.44	0.27	0.04	6.82	9.12	0.17	1.67	0.23	0.09
Chloranthaceae	*Hedyosmum brasiliense*	10.77	12.31	0.60	0.57	0.03	2.28	3.03	0.03	3.23	0.77	0.04
Chrysobalanaceae	*Hirtella hebeclada*	9.14	13.86	0.64	0.52	0.04	2.60	7.73	0.12	3.30	0.34	0.06
Clusiaceae	*Calophyllum brasiliense*	27.41	28.40	11.26	6.63	0.99	20.68	20.75	4.62	1.00	0.21	0.09
Clusiaceae	*Clusia criuva*	5.27	10.30	0.58	0.33	0.06	1.77	4.15	0.25	40.60	0.25	0.11
Clusiaceae	*Garcinia gardneriana*	25.25	30.62	10.38	5.61	0.98	14.80	25.24	4.78	1.24	0.21	0.09
Connaraceae	*Connarus sp.*	6.98	12.07	0.31	0.06	0.03	6.98	11.43	0.26	1.00	0.12	0.10
Dilleniaceae	*Doliocarpus glomeratus*	7.31	6.14	0.19	0.14	0.01	5.00	5.25	0.05	1.23	0.21	0.05
Ericaceae	*Gaylussacia brasiliensis*	7.44	6.70	0.24	0.22	0.03	1.79	2.68	0.02	9.20	1.73	0.12
Erythroxylaceae	*Erythroxylum amplifolium*	5.72	7.48	0.15	0.11	0.07	3.67	6.95	0.04	1.00	1.75	0.49
Euphorbiaceae	*Margaritaria nobilis*	10.99	6.14	0.49	0.23	0.04	4.85	5.56	0.26	4.18	0.15	0.08
Lauraceae	*Cryptocarya moschata*	19.33	21.41	4.24	2.12	0.35	14.20	18.56	2.12	1.00	0.17	0.08
Lauraceae	*Ocotea pulchella*	5.75	8.17	0.15	0.08	0.03	4.60	7.19	0.08	1.00	0.34	0.17
Loranthaceae	*Struthanthus concinnus*	3.98	5.82	0.06	0.04	0.01	2.69	4.44	0.02	1.00	0.55	0.17
Malpighiaceae	*Byrsonima ligustrifolia*	13.57	10.37	1.23	1.05	0.17	6.94	6.40	0.19	1.00	0.89	0.14
Marcgraviaceae	*Norantea brasiliensis*	9.59	6.61	0.32	0.25	0.04	1.47	4.97	0.07	6.45	0.58	0.13

Family	Species	Fruit diameter (mm)	Fruit length (mm)	Fruit fresh mass (g)	Pulp fresh mass (g)	Pulp dry mass (g)	Seed diameter (mm)	Seed length (mm)	Seed mass/ fruit (g)	Seed number	Pulp/seed	RY
Melastomataceae	*Miconia rigidiuscula*	5.67	4.26	0.09	0.08	0.01	1.77	2.39	0.01	3.73	0.91	0.11
Melastomataceae	*Ossaea retropila*	8.77	8.22	0.31	0.16	0.05	0.45	0.63	0.154	539.80	0.32	0.16
Meliaceae	*Cabralea canjerana*	10.21	18.45	1.27	0.70	0.35	8.99	10.79	0.57	1.48	0.62	0.27
Meliaceae	*Guarea macrophylla*	8.79	12.42	0.51	0.15	0.04	8.24	10.88	0.36	1.00	0.11	0.08
Menispermaceae	*Abuta selloana*	16.35	34.30	5.52	2.31	0.39	13.22	32.21	3.21	1.00	0.12	0.07
Menispermaceae	*Cissampelos pareira*	7.31	7.30	0.21	0.17	0.07	4.93	5.61	0.04	1.00	2.01	0.36
Monimiaceae	*Mollinedia schottiana*	7.57	10.33	0.33	0.16	0.05	5.95	8.98	0.17	1.00	0.29	0.15
Moraceae	*Ficus adhatadifolia*	19.84	18.17	2.51	2.49	0.26	1.12	1.24	0.02	156	13.00	0.10
Moraceae	*Ficus arpazusa*	20.86	17.37	3.27	3.257	0.60	1.47	1.62	0.013	115	46.15	0.18
Moraceae	*Ficus insipida*	29.41	27.23	10.24	10.237	1.10	1.5	2.45	0.0025	107	440.00	0.11
Moraceae	*Ficus organensis*	12.57	10.94	1.12	0.92	0.30	1.29	1.72	0.2	182	1.49	0.27
Moraceae	*Ficus pulchella*	24.67	22.66	6.29	5.30	0.80	1.2	1.34	0.99	109	0.80	0.13
Moraceae	*Sorocea bonplandii*	15.91	16.56	2.79	2.15	0.29	9.84	11.76	0.64	1.00	0.45	0.10
Myristicaceae	*Virola bicuhyba*	17.72	28.32	4.28	1.55	0.71	14.70	21.78	2.74	1.00	0.26	0.17
Myrsinaceae	*Cybianthus peruvianus*	7.24	6.45	0.20	0.12	0.02	5.17	5.04	0.08	1.00	0.25	0.10
Myrsinaceae	*Rapanea umbellata*	6.31	5.61	0.13	0.10	0.01	3.68	4.22	0.03	1.00	0.49	0.11
Myrsinaceae	*Rapanea venosa*	6.68	5.48	0.14	0.10	0.03	4.27	4.14	0.05	1.00	0.60	0.21
Myrtaceae	*Campomanesia guaviroba*	35.38	26.61	17.51	15.44	1.13	8.62	11.00	2.07	9.80	0.55	0.06
Myrtaceae	*Campomanesia xanthocarpa*	17.56	14.78	2.72	2.56	0.42	4.90	2.00	0.17	7.33	2.51	0.15
Myrtaceae	*Eugenia cuprea*	14.83	17.71	1.84	1.18	0.36	9.45	11.63	0.66	1.13	0.55	0.20
Myrtaceae	*Eugenia sp.*	20.49	22.15	5.03	2.78	0.43	15.57	14.66	2.25	1.00	0.19	0.09
Myrtaceae	*Eugenia sulcata*	10.19	6.99	0.31	0.16	0.03	7.82	4.44	0.15	1.16	0.18	0.09
Myrtaceae	*Eugenia umbelliflora*	12.12	14.95	1.38	0.78	0.26	9.13	12.06	0.60	1.16	0.43	0.19
Myrtaceae	*Gomidesia fenzliana*	9.21	6.78	0.37	0.32	0.06	3.89	2.91	0.04	2.00	1.50	0.16
Myrtaceae	*Gomidesia flagelaris*	16.29	15.48	2.28	1.59	0.24	11.09	11.23	0.69	1.04	0.35	0.11
Myrtaceae	*Gomidesia schaueriana*	13.52	12.00	1.28	1.09	0.19	5.76	6.42	0.20	1.75	0.96	0.15
Myrtaceae	*Gomidesia spectabilis*	15.91	14.54	2.43	2.00	0.10	9.50	8.61	0.43	1.00	0.23	0.04
Myrtaceae	*Marlieria tomentosa*	17.90	17.09	3.16	1.86	0.31	11.42	12.60	1.30	1.75	0.24	0.10
Myrtaceae	*Myrcia bicarinata*	7.64	6.92	0.26	0.23	0.04	3.89	3.33	0.03	1.00	1.33	0.15
Myrtaceae	*Myrcia insularis*	14.61	12.99	1.68	1.30	0.22	9.59	7.14	0.38	1.00	0.57	0.13
Myrtaceae	*Myrcia multiflora*	5.91	5.22	0.12	0.10	0.02	3.71	3.15	0.02	1.10	1.00	0.17
Myrtaceae	*Myrcia rostrata*	7.56	9.71	0.38	0.28	0.05	4.35	7.52	0.10	1.10	0.55	0.14
Myrtaceae	*Pimenta pseudocaryiophyllus*	7.32	8.08	0.25	0.21	0.06	3.38	4.03	0.03	1.33	1.70	0.22
Myrtaceae	*Psidium cattleyanum*	26.84	26.61	10.71	8.95	1.73	2.59	3.43	1.82	94.72	0.95	0.16
Myrtaceae	*Siphoneugena guilfoyleiana*	9.96	8.41	0.52	0.40	0.10	4.08	5.80	0.13	2.00	0.77	0.19
Nyctaginaceae	*Guapira opposita*	6.53	6.95	0.21	0.16	0.03	3.84	6.08	0.05	1.00	0.47	0.12

Family	Species	Fruit diameter (mm)	Fruit length (mm)	Fruit fresh mass (g)	Pulp fresh mass (g)	Pulp dry mass (g)	Seed diameter (mm)	Seed length (mm)	Seed mass/ fruit (g)	Seed number	Pulp/seed	RY
Olacaceae	*Heisteria silvianii*	9.90	12.25	0.61	0.19	0.03	8.44	11.56	0.42	1.00	0.06	0.04
Olacaceae	*Ximenia americana*	24.74	28.56	10.77	7.81	0.89	16.00	21.80	2.97	1.00	0.30	0.08
Quiinaceae	*Quiina glazovii*	15.91	21.71	2.99	1.45	0.23	11.40	20.27	1.53	1.00	0.15	0.08
Rubiaceae	*Chiococca alba*	5.93	6.15	0.08	0.06	0.01	2.79	4.81	0.02	2.00	0.64	0.13
Rubiaceae	*Coccocypselum cordifolium*	7.47	8.08	0.16	0.15	0.01	1.80	1.91	0.01	14.48	0.81	0.06
Rubiaceae	*Coccocypselum lanceolatum*	8.61	13.62	0.27	0.26	0.015	1.41	1.53	0.01	19.10	1.10	0.05
Rubiaceae	*Geophila repens*	8.61	13.62	0.27	0.26	0.020	1.44	1.53	0.01	19.10	1.46	0.07
Rubiaceae	*Psychotria deflexa*	7.07	5.66	0.12	0.10	0.004	2.69	2.94	0.02	2.00	0.23	0.03
Rubiaceae	*Psychotria hoffmannseggiana*	7.24	5.97	0.16	0.14	0.01	3.16	3.51	0.02	1.60	0.35	0.04
Rubiaceae	*Psychotria kleinii*	14.22	17.13	1.48	1.35	0.04	7.02	7.65	0.13	2.00	0.30	0.03
Rubiaceae	*Psychotria leiocarpa*	8.18	9.50	0.33	0.31	0.00	3.19	5.34	0.02	2.00	0.04	0.00
Rubiaceae	*Psychotria mapourioides*	6.20	5.54	0.15	0.12	0.01	3.43	3.88	0.02	2.00	0.41	0.07
Rubiaceae	*Psychotria nemorosa*	7.16	4.94	0.10	0.09	0.01	2.38	2.50	0.01	1.77	0.75	0.10
Rubiaceae	*Psychotria nuda*	14.45	13.59	1.24	1.14	0.23	3.11	7.86	0.10	1.90	2.41	0.19
Rubiaceae	*Rudgea heurchkii*	12.02	10.08	0.59	0.53	0.03	4.40	5.57	0.06	1.97	0.49	0.05
Rubiaceae	*Rudgea villiflora*	11.01	9.45	0.72	0.59	0.02	5.12	6.66	0.13	2.00	0.15	0.03
Sabiaceae	*Pouteria psammophila*	27.01	26.86	8.97	4.55	0.57	14.99	19.52	4.42	1.93	0.13	0.06
Santalaceae	*Phoradendron hexastichum*	3.83	3.68	0.02	0.02	0.004	2.20	1.33	0.00	1.00	1.28	0.21
Sapindaceae	*Allophylus petiolulatus*	9.60	10.00	0.53	0.39	0.03	6.28	8.05	0.13	1.00	0.23	0.06
Sapindaceae	*Paullinia seminuda*	9.96	9.91	0.53	0.25	0.05	7.96	7.71	0.29	1.00	0.18	0.10
Sapotaceae	*Manilkara subsericea*	18.75	20.08	3.86	3.57	1.18	7.78	14.26	0.29	1.06	4.05	0.31
Sapotaceae	*Pradosia lactescens*	27.26	42.51	17.97	14.74	1.16	13.39	29.58	3.24	1.00	0.36	0.06
Smilacaceae	*Smilax brasiliensis*	7.41	7.33	0.25	0.15	0.02	5.29	5.26	0.10	1.00	0.20	0.08
Smilacaceae	*Smilax quinquenervia*	14.14	14.24	1.60	1.13	0.16	7.59	8.08	0.47	1.93	0.34	0.10
Solanaceae	*Cestrum sp.*	10.39	15.13	0.81	0.66	0.12	3.80	6.85	0.15	4.60	0.80	0.15
Solanaceae	*Solanum capsicoides*	26.17	22.55	6.44	4.75	0.94	5.06	4.71	1.69	241.43	0.56	0.15
Symplocaceae	*Symplocos laxiflora*	12.54	18.92	1.83	1.20	0.27	8.18	16.01	0.64	1.00	0.42	0.15
Theaceae	*Ternstroemia brasiliensis*	3.80	5.87	0.04	0.01	0.0040	3.54	5.83	0.03	1.00	0.15	0.11
Urticaceae	*Coussapoa microcarpa*	10.99	7.73	0.46	0.4580	0.07	1.54	2.57	0.002	37.30	35.00	0.15
Urticaceae	*Pourouma guianensis*	14.84	16.77	2.02	1.18	0.27	9.59	15.68	0.84	1.00	0.33	0.14
Verbenaceae	*Vitex polygama*	16.63	17.06	2.57	1.97	0.48	8.15	13.72	0.59	1.00	0.81	0.19
Vitaceae	*Cissus paullinifolia*	13.59	13.37	1.64	1.32	0.18	8.20	11.63	0.32	1.00	0.56	0.11
Vitaceae	*Cissus verticillata*	6.56	6.22	0.20	0.16	0.02	3.81	5.13	0.04	1.00	0.54	0.10

Appendix III – Chemical characteristics of 78 fruit species from Cardoso Island, Atlantic rainforest, Brazil.

Family	Species	% Water	% Lipids	% Proteins	% Glucose	% Sucrose	% Fructose	Total sugar (glu+su+fru)	Energy (KJ)	% Phenol	% Tannins
Anacardiaceae	*Schinus terebinthifolius*	65.91	5.47	6.86	0.80	-	0.07	0.87	3.52	2.30	0.02
Anacardiaceae	*Tapirira guianensis*	77.94	9.90	4.46	1.53	-	2.00	3.52	5.31	2.18	0.07
Annonaceae	*Guatteria australis*	82.35	6.45	4.87	0.98	-	0.62	1.60	3.69	0.82	0.03
Annonaceae	*Rollinia sericea*	94.04	21.71	11.28	0.01	0.01	0.01	0.03	10.55	2.48	0.02
Aquifoliaceae	*Ilex amara*	79.38	7.89	2.08	0.90	-	1.37	2.27	3.88	0.83	0.02
Arecaceae	*Bactris setosa*	60.19	2.99	5.37	0.29	-	0.12	0.41	2.20	1.17	0.04
Arecaceae	*Euterpe edulis*	66.35	21.67	4.22	0.02	-	0.01	0.03	9.28	1.30	0.03
Arecaceae	*Geonoma schottiana*	79.07	5.43	9.35	0.02	-	0.01	0.03	3.80	4.50	0.17
Arecaceae	*Siagrus romanzoffiana*	57.14	5.07	4.14	1.48	-	1.35	2.83	3.23	1.01	0.03
Boraginaceae	*Cordia verbenacea*	87.50	18.42	9.45	0.84	-	1.25	2.09	9.29	1.06	0.02
Bromeliaceae	*Aechmea nudicaulis*	70.59	1.87	4.56	0.17	-	0.15	0.32	1.60	1.15	0.04
Burseraceae	*Protium heptaphyllum*	80.16	9.85	6.06	0.71	-	0.70	1.41	5.20	1.12	0.02
Celastraceae	*Maytenus robusta*	76.38	1.79	2.63	0.11	-	0.07	0.18	1.20	0.61	0.08
Clusiaceae	*Calophyllum brasiliense*	85.08	4.23	3.66	0.13	-	0.42	0.55	2.41	0.79	0.02
Clusiaceae	*Garcinia gardneriana*	82.47	2.69	4.84	1.54	-	1.44	2.98	2.45	0.78	0.04
Connaraceae	*Connarus sp.*	46.30	66.54	6.31	0.01	-	0.01	0.02	27.28	2.10	0.58
Dilleniaceae	*Doliocarpus glomeratus*	92.89	9.69	5.87	0.90	-	0.92	1.82	5.18	1.02	0.02
Ericaceae	*Gaylussacia brasiliensis*	87.48	5.48	2.52	1.48	-	1.52	3.00	3.13	1.00	0.03
Erythroxylaceae	*Erythroxylum amplifolium*	31.29	26.87	4.59	0.04	0.01	0.02	0.07	11.39	0.68	0.02
Euphorbiaceae	*Margaritaria nobilis*	82.88	3.49	3.61	0.01	-	0.01	0.02	2.02	0.54	0.02
Lauraceae	*Cryptocarya moschata*	83.51	5.96	10.01	0.46	0.18	0.76	1.40	4.37	1.47	0.05
Lauraceae	*Ocotea pulchella*	65.65	60.57	5.19	0.07	-	0.08	0.15	24.76	1.93	0.05
Malpighiaceae	*Byrsonima ligustrifolia*	83.81	1.50	4.03	0.65	-	0.52	1.17	1.51	2.66	0.22
Marcgraviaceae	*Norantea brasiliensis*	83.87	4.36	5.02	0.88	-	1.43	2.31	3.02	1.08	0.07
Melastomataceae	*Miconia rigidiuscula*	87.23	3.01	5.46	0.99	-	0.83	1.82	2.48	1.23	0.04
Melastomataceae	*Ossaea retropila*	68.75	2.97	5.98	0.90	-	0.84	1.74	2.50	0.81	0.04
Meliaceae	*Cabralea canjerana*	50.31	77.55	7.37	0.01	-	0.01	0.02	31.79	0.80	0.02
Meliaceae	*Guarea macrophylla*	73.51	44.08	8.96	0.02	-	0.08	0.10	18.93	0.80	0.02
Menispermaceae	*Abuta selloana*	83.11	15.66	11.83	0.01	-	0.01	0.02	8.26	1.53	0.02
Menispermaceae	*Cissampelos pareira*	56.02	37.30	10.69	0.01	-	0.05	0.06	16.57	4.01	0.12
Monimiaceae	*Mollinedia schottiana*	67.81	45.24	7.58	0.06	-	0.10	0.16	19.16	1.17	0.02
Moraceae	*Ficus arpazusa*	81.25	6.28	5.04	1.21	-	1.12	2.33	3.78	1.84	0.25
Moraceae	*Ficus insipida*	89.25	4.30	7.80	0.05	-	0.03	0.08	3.09	1.01	0.02
Moraceae	*Ficus organensis*	67.57	4.59	3.91	1.40	-	1.27	2.67	2.98	1.04	0.03

Family	Species	% Water	% Lipids	% Proteins	% Glucose	% Sucrose	% Fructose	Total sugar (glu+su+fru)	Energy (KJ)	% Phenol	% Tannins
Moraceae	*Ficus pulchella*	84.90	5.14	4.30	0.11	-	0.10	0.21	2.82	1.01	0.02
Myristicaceae	*Virola bicuhyba*	54.19	56.91	4.30	0.17	-	0.15	0.32	23.19	1.71	0.02
Myrsinaceae	*Rapanea umbellata*	86.18	7.98	2.86	0.08	-	0.02	0.10	3.67	2.88	0.27
Myrsinaceae	*Rapanea venosa*	70.00	5.62	2.60	0.09	-	0.00	0.09	2.69	6.76	0.90
Myrtaceae	*Campomanesia guaviroba*	92.66	11.39	6.01	0.07	-	0.16	0.23	5.59	0.76	0.02
Myrtaceae	*Campomanesia xanthocarpa*	83.72	3.12	4.16	0.69	-	1.43	2.12	2.34	0.98	0.16
Myrtaceae	*Eugenia cuprea*	69.59	14.11	10.32	0.96	-	1.39	2.35	7.80	0.92	0.03
Myrtaceae	*Eugenia sp.*	84.52	5.66	4.75	0.52	-	0.76	1.28	3.30	1.11	0.07
Myrtaceae	*Eugenia sulcata*	82.66	6.62	9.44	0.21	-	0.28	0.49	4.37	1.69	0.03
Myrtaceae	*Eugenia umbelliflora*	66.66	2.83	2.58	1.33	-	1.35	2.68	2.05	1.11	0.04
Myrtaceae	*Gomidesia fenzliana*	81.25	3.40	4.33	1.29	-	1.49	2.78	2.60	1.24	0.19
Myrtaceae	*Gomidesia schaueriana*	82.53	6.33	4.05	0.10	-	0.15	0.25	3.25	3.38	0.12
Myrtaceae	*Gomidesia spectabilis*	95.01	3.60	4.48	1.26	-	1.33	2.59	2.67	1.32	0.03
Myrtaceae	*Marlieria tomentosa*	83.30	9.30	3.92	0.21	0.00	0.84	1.05	4.54	8.22	0.93
Myrtaceae	*Myrcia bicarinata*	82.88	3.48	5.21	0.85	-	0.50	1.35	2.54	0.95	0.03
Myrtaceae	*Myrcia insularis*	83.06	1.61	4.69	1.06	-	1.52	2.58	1.93	1.51	0.25
Myrtaceae	*Myrcia multiflora*	80.00	11.65	5.29	1.34	-	1.63	2.97	6.05	0.67	0.05
Myrtaceae	*Myrcia rostrata*	81.01	5.88	4.92	1.26	-	1.23	2.49	3.63	0.82	0.03
Myrtaceae	*Psidium cattleyanum*	80.62	6.04	3.02	0.07	-	0.34	0.41	2.99	1.26	0.07
Myrtaceae	*Siphoneugena guilfoyleiana*	75.00	5.43	3.79	0.52	-	0.20	0.72	2.94	5.33	0.17
Nyctaginaceae	*Guapira opposita*	84.00	2.27	8.83	0.08	-	0.07	0.15	2.49	1.12	0.02
Olacaceae	*Heisteria silvianii*	86.91	35.36	11.10	0.06	0.00	0.11	0.17	15.90	0.77	0.02
Olacaceae	*Ximenia americana*	88.60	3.92	5.13	0.62	-	0.60	1.21	2.67	1.23	0.05
Quiinaceae	*Quiina glazovii*	84.38	11.80	9.58	0.01	-	0.02	0.04	6.35	1.00	0.04
Rubiaceae	*Coccocypselum cordifolium*	93.11	2.77	4.29	0.59	-	0.21	0.80	2.00	0.60	0.02
Rubiaceae	*Coccocypselum lanceolatum*	94.25	2.70	7.16	1.37	0.17	1.02	2.56	2.78	0.50	0.02
Rubiaceae	*Psychotria kleinii*	97.03	1.71	8.46	0.01	-	0.02	0.04	2.18	0.77	0.02
Rubiaceae	*Psychotria leiocarpa*	99.68	2.19	7.48	1.23	-	1.39	2.62	2.66	0.78	0.02
Rubiaceae	*Psychotria mapourioides*	91.78	2.79	5.15	0.13	-	0.12	0.25	2.06	4.16	0.18
Rubiaceae	*Psychotria nemorosa*	88.59	2.88	9.90	0.99	-	0.71	1.70	3.19	4.83	0.03
Rubiaceae	*Psychotria nuda*	79.84	2.75	7.30	0.96	-	1.09	2.06	2.75	0.64	0.04
Rubiaceae	*Rudgea heurchkii*	94.38	1.97	5.06	1.55	-	2.21	3.75	2.34	0.86	0.03
Rubiaceae	*Rudgea villiflora*	96.61	1.53	3.31	1.73	-	2.04	3.76	1.86	0.57	0.03
Sabiaceae	*Pouteria psammophila*	87.47	9.96	18.31	0.20	-	0.17	0.37	7.24	0.95	0.03

Family	Species	% Water	% Lipids	% Proteins	% Glucose	% Sucrose	% Fructose	Total sugar (glu+su+fru)	Energy (KJ)	% Phenol	% Tannins
Sapindaceae	*Allophylus petiolulatus*	92.31	3.15	5.25	0.89	-	0.17	1.06	2.36	0.86	0.02
Sapindaceae	*Paullinia seminuda*	78.59	3.26	6.07	1.49	-	2.22	3.71	3.02	0.85	0.04
Sapotaceae	*Manilkara subsericea*	66.86	21.77	4.31	0.07	-	0.12	0.19	9.36	0.81	0.02
Smilacaceae	*Smilax brasiliensis*	86.66	3.78	4.47	0.02	-	0.01	0.02	2.29	0.88	0.02
Smilacaceae	*Smilax quinquenervia*	85.84	2.57	10.49	0.23	-	0.91	1.14	3.08	0.67	0.02
Solanaceae	*Solanum capsicoides*	80.21	4.60	12.60	0.04	-	0.01	0.05	4.05	0.88	0.02
Theaceae	*Ternstroemia brasiliensis*	52.34	83.33	3.49	0.00	-	0.00	0.00	33.37	0.52	0.01
Urticaceae	*Pourouma guianensis*	76.71	1.38	7.38	1.20	-	1.55	2.75	2.34	1.22	0.04
Verbenaceae	*Vitex polygama*	75.63	1.60	3.56	0.52	-	1.45	1.97	1.61	2.01	0.06
Vitaceae	*Cissus paullinifolia*	86.38	0.29	3.51	0.44	-	1.11	1.55	1.02	1.02	0.07

Appendix IV – Color characteristics of 58 plant species from Cardoso Island, Atlantic rainforest, Brazil.

Family	Species	Bird chromatic contrast	Bird achromatic contrast	Primate crhormatic contrast	Primate achromatic contrast	Fruit brightness	Fruit Chroma	Fruit hue	Leaf brightness	Leaf chroma	Leaf hue
Anacardiaceae	*Schinus terebinthifolius*	31.1005	2.1519	29.6782	12.2176	627.9586	2.7906	660	467.7566	2.8368	560
Annonaceae	*Guatteria australis*	76.5946	21.8021	20.7978	29.1662	241.3144	1.1878	695	689.1590	1.8270	550
Annonaceae	*Rollinia sericea*	30.7538	15.2612	16.8762	14.6639	630.5889	1.9052	600	313.1370	1.2828	550
Annonaceae	*Xylopia langsdorfiana*	112.6562	87.8182	83.5778	85.9691	3118.5649	1.3155	480	850.3369	3.5577	550
Aquifoliaceae	*Ilex amara*	5.3964	28.3414	5.2290	29.0226	75.9588	2.3594	695	276.4058	1.6430	585
Arecaceae	*Attalea dubia*	22.2628	21.9322	16.1188	19.8162	1166.9561	2.4128	660			
Arecaceae	*Euterpe edulis*	35.0439	20.3384	24.2855	20.8130	220.0878	4.0969	695			
Arecaceae	*Geonoma gamiova*	34.4742	27.1965	20.4478	15.7985	122.4717	0.2186	600			
Arecaceae	*Geonoma pauciflora*	60.5279	16.0282	18.8172	24.4117	216.4885	0.9646	695			
Arecaceae	*Geonoma schottiana*	37.1086	1.4244	18.2410	1.8501	178.5343	1.8346	695			
Arecaceae	*Siagrus romanzoffiana*	22.0074	11.0207	9.4070	8.0071	723.8884	1.8811	640			
Boraginaceae	*Cordia verbenacea*	41.8095	22.1276	35.0152	18.2282	2380.0050	1.4368	660	504.3496	2.5994	550
Bromeliaceae	*Aechmea nudicaulis*	12.5649	5.5747	7.0712	5.2984	659.0906	1.5697	580	576.0553	1.5542	660
Burseraceae	*Protium heptaphyllum*	71.6720	17.5362	36.2834	45.2277	1645.1918	1.2641	580			
Chrysobalanaceae	*Hirtella hebeclada*	154.2421	122.4314	108.6316	135.9858	0.7724	5.4492	350	111.4878	5.1266	550
Clusiaceae	*Calophyllum brasiliense*	10.1752	1.6241	1.8319	0.5358	600.5284	1.7136	570	529.1940	1.6407	550
Clusiaceae	*Garcinia gardneriana*	50.7187	32.0515	31.6677	32.0662	793.8718	2.0428	600	165.7559	1.6111	550
Ericaceae	*Gaylussacia brasiliensis*	50.1554	35.7198	42.9246	40.0224	106.1380	0.8830	695	363.2762	3.4648	540
Erythroxylaceae	*Erythroxylum amplifolium*	39.3622	3.5936	13.7590	7.7603	365.5825	2.7359	660	144.8539	6.0548	550
Euphorbiaceae	*Margaritaria nobilis*	69.2169	4.2216	23.5379	4.2110	461.8517	1.1603	480			
Lauraceae	*Ocotea pulchella*	56.0893	60.4950	38.0652	61.6005	20.3148	16.4192	695	42.2113	6.0845	550
Malpighiaceae	*Byrsonima ligustrifolia*	21.2102	19.1902	9.4687	17.9816	118.7822	3.1491	660	176.8404	3.8479	550
Melastomataceae	*Miconia rigidiuscula*	15.2384	7.5653	12.7058	9.6804	354.9410	0.9735	695	425.7976	2.5815	550
Melastomataceae	*Ossaea retropila*	12.6245	6.2112	10.8066	9.0465	332.8264	0.5355	600	399.5025	1.3355	580
Meliaceae	*Cabralea canjerana*	45.9825	7.5864	28.0370	23.6413	983.5362	2.1686	660	279.1827	2.5641	550
Meliaceae	*Guarea macrophylla*	126.1524	12.2642	54.1675	34.3281	1401.0604	3.2289	660	217.9134	3.6648	550
Menispermaceae	*Cissampelos pareira*	30.8595	1.0121	12.3171	4.6501	560.0517	2.1172	660			
Moraceae	*Ficus insipida*	6.7153	13.1142	12.3872	12.7738	607.1680	2.0639	550			
Moraceae	*Ficus organensis*	29.9348	1.3582	7.1185	2.3511	346.9423	2.7966	660	229.2532	1.2401	550
Moraceae	*Sorocea bonplandii*	30.3568	17.0258	24.8995	20.8033	210.7646	0.7383	695	348.3408	3.6124	550
Myristicaceae	*Virola bicuhyba*	38.7192	1.7741	11.0549	7.2562	781.3063	3.3344	660			
Myrsinaceae	*Rapanea umbellata*	52.4322	22.6161	33.3065	26.8548	455.8531	0.9471	695	836.3043	3.3112	560
Myrsinaceae	*Rapanea venosa*	43.4098	46.2182	8.0005	47.8880	45.3146	3.5272	580	415.6653	3.8334	570
Myrtaceae	*Campomanesia xanthocarpa*	12.1210	11.1603	3.5758	8.3452	696.1679	1.6734	635			
Myrtaceae	*Eugenia cuprea*	25.5517	0.8449	14.1000	5.6110	508.7319	2.2375	660			

Family	Species	Bird chromatic contrast	Bird achromatic contrast	Primate crhormatic contrast	Primate achromatic contrast	Fruit brightness	Fruit Chroma	Fruit hue	Leaf brightness	Leaf chroma	Leaf hue
Myrtaceae	*Eugenia sp.*	14.1070	8.1558	6.6509	5.1807	629.5459	1.7772	660			
Myrtaceae	*Eugenia sulcata*	29.0554	18.1333	25.1057	21.7340	158.0662	0.6839	695	267.4168	3.7426	550
Myrtaceae	*Myrcia insularis*	111.3107	30.4364	90.1620	34.9404	47.1883	3.5718	695	103.3544	4.7823	550
Myrtaceae	*Myrcia multiflora*	31.2845	15.7066	23.6019	18.7855	171.2224	2.6417	695	248.8190	5.4043	560
Myrtaceae	*Myrcia rostrata*	155.0272	119.0054	117.4960	135.3572	0.9571	3.6365	335	87.0203	6.0067	550
Myrtaceae	*Pimenta pseudocaryiophyllus*	11.0561	9.3564	9.3138	11.1731	233.3039	0.9617	695	299.2386	1.0399	550
Myrtaceae	*Psidium cattleyanum*	44.7067	18.3784	8.1431	17.1020	947.8510	1.5365	610			
Nyctaginaceae	*Guapira opposita*	117.2790	32.8226	68.8155	43.0334	131.0173	2.2629	580	97.4263	5.4106	550
Olacaceae	*Heisteria silvianii*	74.1430	19.3918	80.9042	26.2322	992.1217	1.3342	510	703.9737	3.0569	560
Olacaceae	*Ximenia americana*	24.6664	10.6011	10.8165	8.4543	852.8453	1.6582	600	404.9103	3.6933	550
Rubiaceae	*Geophila repens*	18.3138	9.0495	6.6349	7.3632	257.0603	2.6425	610	158.4866	3.5516	585
Rubiaceae	*Psychotria kleinii*	88.6296	5.0815	91.3250	11.2514	406.0623	4.7282	480	185.5358	5.3536	550
Rubiaceae	*Psychotria nuda*	88.2626	3.0807	107.0476	20.7675	786.0756	4.5529	485	291.0682	4.1462	550
Sapindaceae	*Paullinia seminuda*	15.2766	3.2889	13.4350	37.5925	1066.7446	1.0727	635	481.4097	2.4621	560
Sapotaceae	*Pradosia lactescens*	11.0072	11.0255	5.8871	8.6266	689.9008	1.8915	660			
Smilacaceae	*Smilax brasiliensis*	85.2219	62.9872	15.0553	69.6858	18.1045	6.3401	695	210.2739	6.8134	550
Smilacaceae	*Smilax quinquenervia*	43.3746	14.4364	86.0369	17.1334	194.6134	3.0495	660			
Solanaceae	*Cestrum sp.*	9.8749	5.8368	8.5734	7.5439	376.2527	0.8673	695	428.9582	0.7782	550
Solanaceae	*Solanum capsicoides*	35.5044	9.8087	24.5164	14.0195	818.6448	2.8884	660	843.7537	4.1357	550
Theaceae	*Ternstroemia brasiliensis*	46.3565	13.6331	14.8836	6.2712	1227.0301	3.6656	660			
Urticaceae	*Coussapoa microcarpa*	16.1885	4.9297	6.1876	3.6583	502.4351	1.6052	580	373.5833	2.1297	550
Urticaceae	*Pourouma guianensis*	43.3746	18.0579	27.9588	21.9043	216.6471	0.4299	695			
Vitaceae	*Cissus paullinifolia*	30.9738	21.7683	21.2963	25.8528	372.3696	3.1383	660	639.4335	3.2714	560