8 Secondary Metabolites of Ripe Fleshy Fruits: Ecology and Phylogeny in the Genus *Solanum*

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Introduction and Overview

Why do ripe fleshy fruits contain secondary metabolites, sometimes in concentrations that make the fruits toxic to vertebrates? And how do the chemical and physical characteristics of fruits influence choice by frugivores? These questions have drawn the attention of those interested in understanding the adaptive roles of fruit traits (see Cipollini and Levey, 1997a, b,c; Cipollini, 2000). However, a criticism of such studies is that few have addressed the possible influence of physiological and phylogenetic constraints on fruit traits (see Cipollini and Levey, 1998; Eriksson and Ehrlen, 1998). We are addressing this concern by studying fruit traits of the genus *Solanum*, while assessing and accounting for phylogenetic effects. Here we provide a preliminary analysis of our data, which we hope will stimulate others to conduct similar analyses.

Theoretically, predictable differences in the quality of seed dispersal among frugivores should influence the evolution of fruit traits (see Janson, 1983; Wheelwright, 1985; Jordano 1987a; Debussche and Isenmann, 1989; Willson *et al.*, 1989; Gautier-Hion, 1990; Stiles and Rosselli, 1992). Nevertheless, studies using phylogenetic null models have not supported this theory (see Bremer and Eriksson, 1992; Herrera, 1992; Jordano, 1995). This lack of support is assumed to result, in part, because of extensive decoupling of interactions between particular frugivores and the plants whose seeds they disperse (see Howe, 1984; Jordano, 1987b; Herrera, 1998). However, most studies on the evolution of fruit traits have overlooked the effects of secondary metabolites, which are probably important mediators of fruit–frugivore interactions (Cipollini and Levey, 1997c, 1998).

We have presented several adaptive hypotheses on the adaptive significance of secondary metabolites in ripe fleshy fruits (Cipollini, 2000). In general, these hypotheses assume a selective advantage to plants bearing fruits containing secondary metabolites and thus predict that patterns of secondary chemistry in fruits can be explained, at least in part, by interactions with frugivores. Likewise, the hypotheses

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predict that fruit-use patterns by frugivores can be explained, at least in part, by the presence of secondary metabolites. The putatively adaptive pattern upon which we focus is an apparent correlation of ripe-fruit glycoalkaloid (GA) (potentially toxic metabolite) content with other fruit traits, a pattern that could result from diffuse coevolution leading to broadly defined seed-dispersal syndromes (suites of species showing similar, but independently evolved, fruit characteristics (*sensu* van der Pjil, 1969)). In particular, ripe fruits of *Solanum* species that are dispersed by birds have very low GA levels; those dispersed by bats have variable levels; and those dispersed by terrestrial mammals have high levels (Cipollini and Levey, 1997c). Such patterns may have resulted from coadaptive fruit–frugivore interactions. Or they might simply reflect physiological constraints related to leaf and unripe-fruit defence and/or the effects of shared ancestry. For example, Ehrlen and Eriksson (1993) postulated that 'toxic' fruits are more common in related plant taxa and that ripe-fruit toxicity primarily results from physiological constraints associated with selection for defences in leaves and/or unripe fruits (see also Eriksson and Ehrlen, 1998). Considering these alternatives, we focus on two questions.

1. Are ripe *Solanum* fruits that are high in GAs primarily found in species:
(a) whose leaves are high in GAs?
(b) whose unripe fruits are high in GAs?
(c) whose closest relatives produce fruits high in GAs?
2. Are patterns of fruit-trait covariation consistent with independent selection pressures, or are patterns associated strongly with phylogeny?

General Phylogenetic Approach

To answer our two questions, we are collecting molecular, physical and chemical data for a large group of *Solanum* species whose fruit traits contrast markedly. By using techniques that account for phylogeny, we hope to provide rigorous, phylogenetically corrected statistical tests of the relationships among traits (e.g. the correlation between leaf and fruit chemical traits). This is, to our knowledge, the first use of molecular data as a basis for a comparative study of fruit chemistry in wild plants (see Bremer and Eriksson (1992) for an analysis of fruit morphology).

A primary requirement of our approach is a rigorous phylogenetic hypothesis for our species. To avoid circularity, this phylogeny should be derived independently of the fruit traits we are examining (Givnish, 1997; but see de Queiroz, 1996). Our approach is thus to determine a phylogeny for our species using gene sequence data and to measure a suite of fruit traits considered to be relevant to frugivores. We then use the methods of independent contrasts (IC), phylogenetic autocorrelation (PA), and signed Mantel (MAN) tests to control for species relatedness in statistical tests and to examine whether predicted patterns exist among the traits after the effects of phylogeny have been removed or minimized.

Study Species and Taxonomic Background

The genus *Solanum* was selected primarily because traits relating to seed dispersal vary tremendously in this group. It is one of the largest plant genera (*c.* 1400 species) and encompasses remarkable diversity in morphology, habit and distribution. The most widely used infrageneric classification divides *Solanum* into seven subgenera and about 70 sections (D'Arcy, 1972, 1991). Some sections have been the subject of intensive study because of their economic importance (e.g. the potatoes (section *Petota*)) and others have been the focus of recent taxonomic revisions and/or phylogenetic studies (see Olmstead and Palmer, 1997; Olmstead *et al.*, 1999; Spooner *et al.*, 1999). While many groups are poorly known, a consistent picture is emerging of phylogenetic relationships in the genus as a whole, and progress has been made in defining monophyletic sections (see Bohs and Olmstead 1997, 1999). Analyses of sequence data from the chloroplast gene *ndhF* and the nuclear ITS (internal transcribed spacer) region identify about 11 major clades in the genus (Bohs, 2000). Some of these clades are congruent with traditional taxonomic

subdivisions, whereas others are not. Our preliminary approach has been to sample species from disparate clades as a means of obtaining a large sample that is diverse in fruit traits.

Solanum GAs: Ecological and Phylogenetic Context

Total glycolalkaloid (TGA) content

We focus on TGA, because studies suggest that quantitative variation probably overrides most differences in the deterrent effects of specific GAs toward consumers. For example, Cipollini and Levey (1997a,b,c) found little difference in the deterrence of the two most prevalent GAs (solamargine and solasonine) towards a wide variety of organisms. The potato GAs (solanine and chaconine) show similar general deterrent effects (see van Gelder, 1990), although some variation in toxicity among organisms has been noted, depending on the specific compound tested. Moreover, the patterns that we are examining are principally quantitative, especially regarding correlations of putatively physiologically constrained traits within plant species (e.g. leaf vs. ripe-fruit GA).

Ecological patterns of Solanum *fruit GAs*

Among temperate North American *Solanum*, only the large, low-nutrient, yellow, odorous, winter-dispersed, mammal-syndrome fruit, *S. carolinense*, is known to contain high concentrations of GAs when ripe (Cipollini and Levey, 1997a,b,c). This suggests that ripe-fruit toxins might defend against pests when dispersal is rare or unpredictable, and thus may represent a trade-off between defence against pests and palatability for dispersers. In a study of other *Solanum*, including tropical species, this finding was corroborated; high levels of GAs were commonly found in fruits having traits suggesting dispersal by terrestrial mammals (Cipollini and Levey, 1997c). All 'bird' fruits (small, high-nutrient, red, black, odourless) showed little or no detectable GA, whereas 'bat' fruits (variable-sized, high-nutrient, dull green or yellow-green, strong odours) had variable levels. Nee (1991) likewise suggested an association of high GA content with some mammal-dispersed species. High GA content in terrestrial mammal-dispersed fruits may be possible because the large body size of such mammals may confer some tolerance to GAs (van Gelder, 1990). Plants dispersed by such animals might more easily afford to protect their fruits from pests via high levels of GAs. Consumption of such fruits might even reduce parasitic infection in some species (e.g. the maned wolf, *Chrysocyon brachyurus*, which feeds on *Solanum lycocarpon* (Courtenay, 1994)).

Known phylogenetic patterns

While much is known about *Solanum* GAs in leaves and fruits (see Schreiber, 1968; Ripperger and Schreiber, 1981), little use has been made of these data from an evolutionary perspective. Based upon a few studies, we note the following patterns.

1. In a reanalysis of data collected by Bradley *et al.* (1979) on 47 *Solanum* species, we found a significant correlation between leaf and unripe-fruit GA concentration ($r = 0.58$, $P \leq 0.0001$). However, 16 species with no GAs in their leaves had significant levels (0.1–1.5% dry weight) in the fruits. Because few ripe fruits were analysed, however, these data are inadequate to rigorously test for a correlation between leaf and ripe-fruit GA content.
2. While many *Solanum* fruits lose GAs with ripening (e.g. Schreiber, 1963; Bradley *et al.*, 1979), plants with 'toxic' ripe fruits are found in at least three subgenera and at least ten groups or sections of the genus (Schreiber, 1963; Kingsbury, 1964; Bradley *et al.*, 1979; M.L. Cipollini, D. Levey, E. Paulk, K. Mink and L.A. Bohs, unpublished data). These results suggest that fruit toxicity is relatively unconstrained by phylogeny.
3. Using published data, we conducted nested analysis of variance of unripe- and ripe-fruit TGA based upon a widely accepted taxonomy of the genus (D'Arcy, 1991). These analyses indicated that a significant amount of variation in TGA content resides among species within sections and hence could be

adaptive (77.6% and 35.2% of variation for unripe and ripe fruits, respectively). This suggests only weak effects of phylogeny on fruit chemistry, provided that D'Arcy's (1991) taxonomic scheme accurately represents phylogenetic relationships within the genus.

Methods

Species selection, growth and sample collection

To avoid biases found in the literature, we collect all data from greenhouse-grown plants using standard molecular and phytochemical methods. We obtained seeds of about 90 *Solanum* species and are growing these plants in common garden conditions (voucher data for species used in this chapter are given in Table 8.1). Seeds were collected from field sites and from the Botanical Garden at Nijmegen, The Netherlands. After growing plants to maturity, we collect about 100 g of both unripe and ripe fruits and 20–30 leaves.

Fruit separation and morphological analyses

For ripe-fruit samples, we measure the following morphological traits: whole-fruit wet mass, pulp and seed wet and dry masses, seed and pulp dry-matter content, seed number per fruit and mean individual dry seed mass. Ripe-fruit colour is recorded by classifying fruits as black/purple, red, orange, yellow, white and/or green.

Chemical analyses

Immature and mature fruits

We analyse freeze-dried pulp samples for total protein using the Bradford assay (Jones *et al.*, 1989), for total soluble sugars using the anthrone technique (Smith, 1981) and for total phenolics using the Prussian blue method (Budini *et al.*, 1980). Based upon preliminary data, total lipids are assumed to be low and unimportant.

GA of fruits and leaves

Freeze-dried leaf and fruit-pulp samples are analysed for GA (TGA analysis) using the Birner (1969) technique.

Molecular analyses

DNA was extracted from fresh or silica-dried leaves using the modified CTAB procedure of Doyle and Doyle (1987) or by a mini-extraction protocol (available upon request from Lynn Bohs). The phylogenies we use are based on sequence data from the nuclear ITS region (ITS 1, ITS 2 and the intervening 5.8S ribosomal DNA (rDNA) subunit) (Baldwin *et al.*, 1995). Polymerase chain reaction (PCR) amplification, clean-up of DNA and PCR products, sequencing and sequence editing and alignment followed the techniques described in Bohs and Olmstead (2001). The data matrix containing the aligned sequences is available from Lynn Bohs upon request.

Phylogenetic analyses were performed using PAUP* 4.0b3a (Swofford, 2000). The parsimony analyses reported here used the heuristic search algorithm with the TBR and MulTrees options, equal weights for all nucleotide positions, gaps treated as missing data and 100 random-order entry replicates. Trees were rooted using *Lycianthes heteroclita* as the outgroup. This species was subsequently deleted from the tree file used in the PA, IC and MAN tests (see below). When multiple, most-parsimonious trees resulted from the searches, a single representative tree was used in the PA, IC and MAN analyses.

Statistical analyses involving fruit traits

TIPS parametric analyses

We first conducted the following parametric analyses using raw data for all taxa under the assumption that the taxa are independent (TIPS analyses). TIPS analyses allow comparisons with the results of phylogenetically corrected analyses using the same data (PA, IC and MAN tests, below); patterns that disappear upon phylogenetic correction via these

Table 8.1. Names of species included in phylogenetically corrected (PA, IC and MAN) analyses, including raw data for ripe-fruit colour, mean whole-fruit wet mass (g) and mean seed number per fruit.

Species	Voucher*†	Colour	Mass	Seed
Lycianthes heteroclita (Sendtn.) Bitter	Bohs 2376	n/a	n/a	n/a
Solanum abutiloides (Griseb.) Bitter & Lillo	RGO S-73 (DNA)/Cipollini 94 (fruits)	O	1.5	199
Solanum acerifolium Dunal	Bohs 2714	G/W	0.7	41
Solanum aculeatissimum Jacq.	Cipollini 60 (DNA)/Cipollini SK (fruits)	Y	4.6	91
Solanum adhaerens Roem. & Schult.	Bohs 2473 (DNA)/Cipollini SL (fruits)	O	–	–
Solanum aphyodendron Knapp	RGO S-92 (DNA)/Cipollini SAP (fruits)	G/Y	–	–
Solanum capsicoides All.	Bohs 2451 (DNA)/Cipollini 37 (fruits)	O	15.3	117
Solanum carolinense L.	Cipollini SC	Y	2.1	103
Solanum cordovense Sessé & Moc.	Bohs 2693	B	0.4	–
Solanum dasyphyllum Schum. & Thonn	Cipollini 7	Y/G	15.0	50
Solanum diflorum Vell.	Cipollini 11	R/O	2.5	85
Solanum dulcamara L.	No voucher (DNA)/Cipollini SD (fruits)	R	0.4	25
Solanum glaucophyllum Desf.	Cipollini 125	B	2.4	17
Solanum jamaicense Mill.	RGO S-85 (DNA)/Bohs 2481 (fruits)	O	0.3	77
Solanum laciniatum Ait.	Bohs 2528	O/Y	1.5	91
Solanum macrocarpon L.‡	Cipollini 101	Y	28.8	405
Solanum mammosum L.	RGO S-89 (DNA)/Cipollini 40 (fruits)	O/Y	22.5	33
Solanum melongena L.	RGO S-91 (DNA)/Cipollini 85 (fruits)	B/Y	18.1	348
Solanum myriacanthum Dunal	Cipollini 83	Y/G	12.7	118
Solanum nigrum L.	Bohs 2534	B	0.2	20
Solanum opacum A. Braun & Bouché	Bohs 2459	G	0.2	35
Solanum physalifolium Rusby	Bohs 2467	G	0.2	20
Solanum prinophyllum Dunal	Bohs 2725	G	–	–
Solanum pseudocapsicum L.	Cipollini 95	R/O	2.6	6
Solanum ptychanthum Dunal	RGO S-94 (DNA)/Cipollini SP (fruits)	B	0.3	55
Solanum rudepannum Dunal	Bohs 2712 (DNA)/Cipollini SRD (fruits)	Y	–	–
Solanum rugosum Dunal	Bohs 3011 (DNA)/Cipollini SRG (fruits)	Y/G	–	–
Solanum scabrum Mill.	Bohs 2729	B	1.3	89
Solanum sciadostylis (Sendtn.) Bohs	Bohs 2453	Y/G	2.5	91
Solanum sessilistellatum Bitter‡	Cipollini 54	–	–	–
Solanum terminale Forssk.	Cipollini 134	R	0.3	13
Solanum torvum Swartz‡	Cipollini 64	Y	–	–
Solanum tucumanense Griseb.	Cipollini 25	R/O	0.8	60
Solanum umbellatum Mill.	Bohs 2560	Y/G	–	–
Solanum variabile Mart.‡	Cipollini 84	O	0.7	21
Solanum viarum Dunal	Cipollini 67	Y	4.9	30
Solanum villosum Mill.	Bohs 2553	O	0.3	43
Solanum virginianum L.	Cipollini 17	Y	4.1	268

‡Determination provisional.

*Bohs vouchers deposited at University of Utah; Cipollini vouchers and Berry College; RGO vouchers at University of Washington.

†If one voucher listed, DNA and fruit analyses were done on same accession. If two vouchers are listed, DNA and fruit analyses were performed on different accessions.

O, orange; G, green; W, white; Y, yellow; B, black/purple; R, red.

methods are assumed to be associated with phylogeny.

REGRESSIONS To provide ahistorical tests of hypotheses relating to physiological constraints, we calculated the linear regressions of fruit TGA content on leaf TGA content. We likewise examined linear regressions of ripe-fruit traits on unripe-fruit traits, and among ripe-fruit traits. Regression parameters were estimated using SPSS for Windows (SPSS, Inc., 1999).

PRINCIPAL COMPONENTS ANALYSIS (PCA) To describe overall relationships among fruit traits and thus test for fruit-trait covariation consistent with fruit-dispersal syndromes, we conducted PCAs using fruit-trait data using SPSS for Windows (SPSS, Inc., 1999). We conducted three TIPS-based PCAs using the following groups of species: (i) 26 species with both fruit chemical and morphological data; (ii) 30 species with both fruit chemical and DNA data; and (iii) 26 species with both fruit morphological and DNA data. Factor loadings were used to determine the strength of association of each fruit trait with each factor. Results using raw and orthogonally rotated matrices were similar and we thus report results only for unrotated matrices.

Phylogenetically corrected analyses

Following TIPS analyses, we applied three types of phylogenetic correction to our data (PA, IC and MAN) and then repeated regression and PCAs. Analyses were performed on data sets for which DNA data were available (sets ii and iii, above). We used all three methods because all are designed to control for inflated degrees of freedom in statistical analyses resulting from the non-independence of related species, and because none is universally accepted as the best approach to phylogenetic correction (Harvey and Pagel, 1991).

PHYLOGENETIC AUTOCORRELATION (PA) ANALYSIS Using one of the most parsimonious molecular phylogenies, we used COMPARE 4.3 (Martins, 2000) to quantify the phylogenetic component of each fruit trait in a PA analysis (Cheverud *et al.*, 1985; Gittleman and Kot, 1990). We then used the specific residual (putatively adaptive) component of each trait value in regression and PCA analyses.

INDEPENDENT CONTRASTS (IC) The IC method controls for phylogeny by splitting trait variation among related species into independent parts (Felsenstein, 1985). This is done via the estimation of nodal values from the phenotypes of descendants below nodes in a phylogeny. Comparisons are made using $(n-1)$ independent standardized contrasts from a data set of n species. So, using COMPARE 4.3 (Martins, 2000) and the same molecular phylogeny used in PA analysis, we generated IC contrasts for each fruit trait and used these contrasts in regression and PCAs. Because the sign of IC contrasts is arbitrary, we computed regressions through the origin when evaluating relationships among traits.

SIGNED MANTEL (MAN) TESTS In signed MAN tests the dissimilarity between pairs of species in a dependent variable (Y variable) is compared with their phylogenetic distance and with their dissimilarity in the other independent variables (X variables) (Legendre *et al.*, 1994; Böhning-Gaese and Oberrath, 1999; Böhning-Gaese *et al.*, 2000). The Y matrix is regressed on the X matrices and tested for significance using MAN tests (Mantel, 1967; Smouse *et al.*, 1986). MAN tests use Monte Carlo randomizations, whereby the X matrices are held constant and the species in the Y matrix are randomly permuted (Smouse *et al.*, 1986; Legendre *et al.*, 1994). This method is a statistical approach to testing the effect of phylogeny on the Y variable and on the relationship between the Y and the X variables. MAN does not assume any particular microevolutionary process except that the mean dissimilarity of any pair of species in a Y variable is a linear function of their phylogenetic distance. To be consistent with TIPS-, IC- and PA-based PCAs, we conducted MAN-based PCAs using the chemical or morphological dissimilarity variables along with the genetic distance values. PCA factor loadings thus correspond to the chemical and morphological variables as well as genetic distance.

Results

Phylogenetic analyses

Fruit chemistry study

The data matrix for the fruit chemistry study included 30 *Solanum* species plus the outgroup *L. heteroclita*. The total aligned length of the sequences was 675 characters, including gaps; of these, 451 were invariant, 224 were variable and 152 were parsimony-informative. Parsimony analysis resulted in one most parsimonious tree of 528 steps (Fig. 8.1), with a

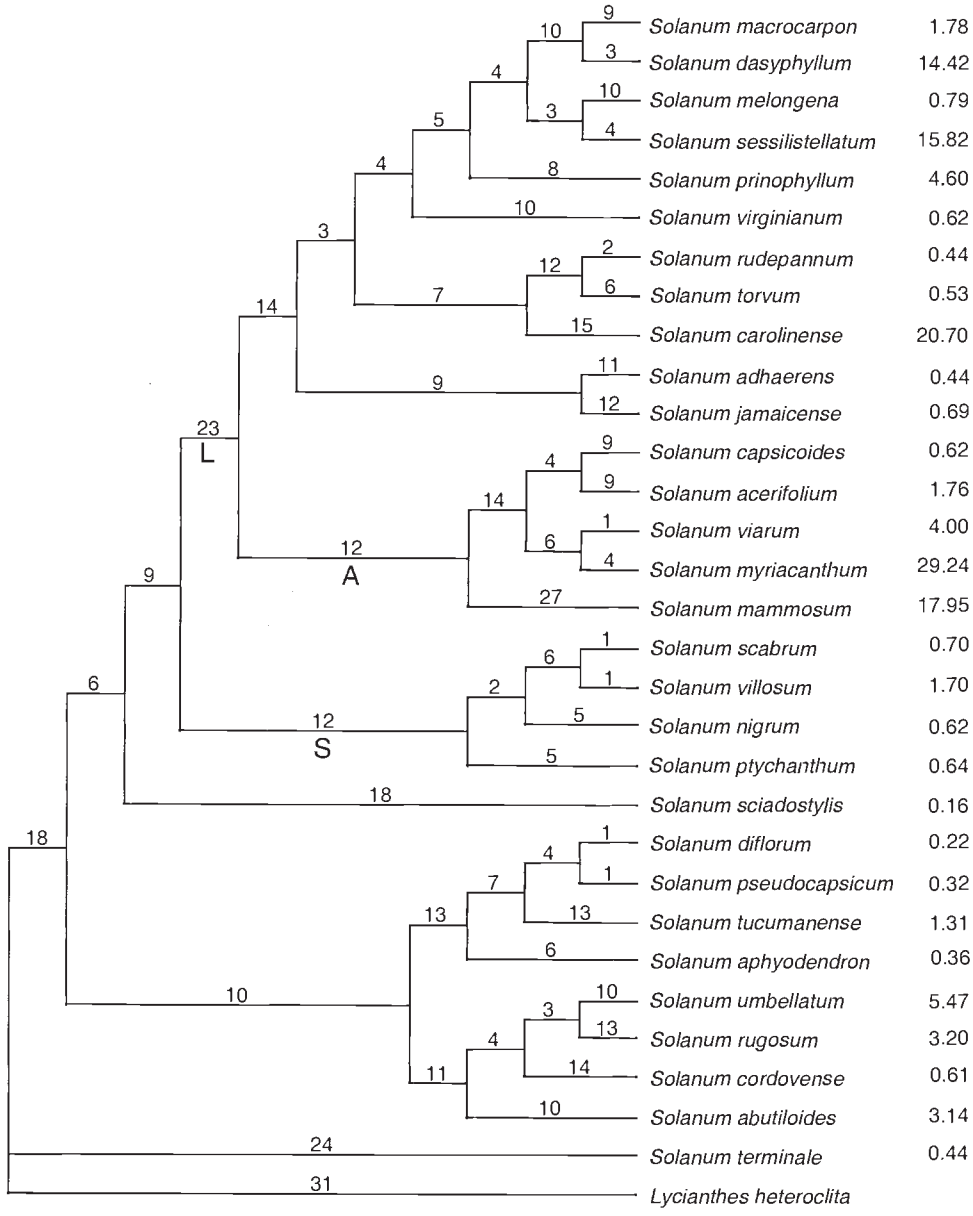

Fig. 8.1. Single most parsimonious tree resulting from analysis of nuclear ITS sequence data for 30 taxa of *Solanum* plus outgroup *Lycianthes heteroclita*. Length = 528 steps, consistency index (CI) excluding uninformative characters = 0.514, retention index (RI) = 0.743. Branch lengths are numbers of nucleotide substitutions; all characters weighted equally and gaps treated as missing data. Branches marked with letters delimit the following monophyletic groups: L = *Solanum* subgenus *Leptostemonum*; A = *Solanum* section *Acanthophora*; S = *Solanum* section *Solanum*. Data following species names are mean values for TGA of ripe-fruit pulp (mg g^{-1} dry mass).

consistency index (CI) (excluding uninformative characters) of 0.514 and a retention index (RI) of 0.743.

Looking across the phylogenetic tree in Fig. 8.1, fruit GA levels vary widely within some clades but are relatively similar in others. For instance, GA levels among species in the subgenus *Leptostemonum* (clade L in Fig. 8.1) vary by over an order of magnitude. This variation is especially notable in section *Acanthophora* (clade A), where fruits of *Solanum myriacanthum* contained nearly 50 times the concentration of those of *Solanum capsicoides*. On the other hand, GA levels among the sampled species of section *Solanum* (clade S) appear to be evolutionarily conservative. This pattern requires further investigation by including data from more species within these clades, and by confirming that GA content does not vary significantly within species.

Fruit morphology study

The fruit morphology data set contained ITS sequence data for 26 *Solanum* species plus the outgroup, *L. heteroclita*. The aligned sequence matrix included 682 characters per taxon (including gaps), of which 442 were invariant and 150 were parsimony-informative. Parsimony analysis found 60 equally parsimonious trees of 534 steps, with a CI (excluding uninformative characters) of 0.521 and an RI of 0.696. One of the 60 most parsimonious trees (not shown) was randomly chosen for input into the PA, IC and MAN tests.

Regression analyses

TIPS regressions indicated no relationship between leaf TGA and either unripe ($R^2 = 0.001$, $P > 0.05$, $n = 18$) or ripe fruit TGA ($R^2 = 0.064$, $P > 0.05$, $n = 18$). In contrast to our reanalysis of Bradley *et al.*'s (1979) data, these results show no evidence for physiological constraints of leaf chemistry on fruit chemistry, albeit for a smaller data set.

TIPS regressions for 30 species with fruit chemical data indicate significant relationships between unripe- and ripe- fruit TGA and between other unripe- and ripe-fruit variables (e.g. total phenolics and proteins (Table 8.2A)). Reanalysis using IC, PA and MAN methods left these results relatively unchanged

Table 8.2. Summary of TIPS-, IC-, PA- and MAN-based regressions for fruit chemical and morphological traits. TIPS results are the R^2 of regressions using raw data for species for which DNA data were available, IC results are the R^2 of regressions through the origin for standardized contrasts for each trait, PA results are the R^2 of regressions of specific residuals derived from phylogenetic autocorrelation analysis and MAN results are the R^2 values for whole-model regressions incorporating the genetic distance matrix as a covariate. All chemical data were in mg g^{-1} dry mass.

Regression	TIP	IC	PA	MAN[a]
A. Fruit chemical traits ($n = 30$ species)				
TGA ripe on unripe	0.263***	0.411***	0.265***	0.164**; ns
Phenolics ripe on unripe	0.148*	0.088ns	0.149*	0.068***; ns
Proteins ripe on unripe	0.408***	0.605***	0.413***	0.220***; ns
TGA ripe on phenolics ripe	0.256***	0.538***	0.257***	0.174***; ns
B. Ripe fruit morphological traits ($n = 26$ species)				
Pulp wet mass (g) on seed wet mass (g)	0.644***	0.751***	0.620***	0.497***; ns
Pulp dry mass (g) on seed dry mass (g)	0.794***	0.638***	0.792***	0.717**; ns
Pulp dry mass (g) on seed number	0.413***	0.347**	0.414***	0.334ns; ns
Seed dry mass (g) on yellow	0.199*	0.020ns	0.145*	0.059**; ns
Seed number on yellow	0.177*	0.028ns	0.172*	0.039*; ns

[a] *P* value for the partial regression coefficient of the dependent variable on the independent variable, followed by the *P* value for the partial regression coefficient of the dependent variable on the genetic distance matrix.

ns, $P > 0.05$; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

(only occasionally was the significance of a relationship lost). This suggests that, for fruit chemical variables, phylogenetic effects are negligible and hence a correlation between unripe- and ripe-fruit secondary chemistry is supported. The magnitude of these effects is weak, however, as evidenced by relatively low R^2 values (Table 8.2A). Results of all regression analyses also suggest that, independent of phylogeny, fruits high in TGAs tend also to be high in phenolics (Table 8.2A).

TIPS regressions for fruit morphological traits were also relatively unaffected by phylogenetic correction (Table 8.2B). In all analyses, positive relationships were found between seed mass, pulp mass and seed number, indicative of allometric relationships among the traits. Seed number and seed mass were also positively related with yellow fruit colour (Table 8.2B; the relationship between yellow colour and whole-fruit mass was marginally significant (results not shown)). These and the regression of ripe-fruit phenolics on unripe-fruit phenolics were the only analyses where the IC method gave results incongruent with TIPS, PA or MAN analyses (being non-significant). Apart from those regressions in Table 8.2, we found no other significant regressions among fruit traits.

PCA analyses

Fruit morphology and chemistry

TIPS-based PCA using 26 species with fruit chemical and morphological traits shows a pattern consistent with preliminary expectations about overall fruit-trait variation (Fig. 8.2; Table 8.3A). In particular, the first PCA axis (which accounted for 28.85% of total variation) differentiates high-GA, high-phenolic, low-protein, low-sugar, large, dense (high dry-matter content), yellow fruits (mammal syndrome?) from low-GA, low-phenolic, high-protein, high-sugar, small, watery (low dry-matter content), red and black fruits (bird syndrome?). The second axis (14.68% of total variation) further differentiates yellow fruits with high TGA, high phenolics and large seed mass from larger orange and red fruits.

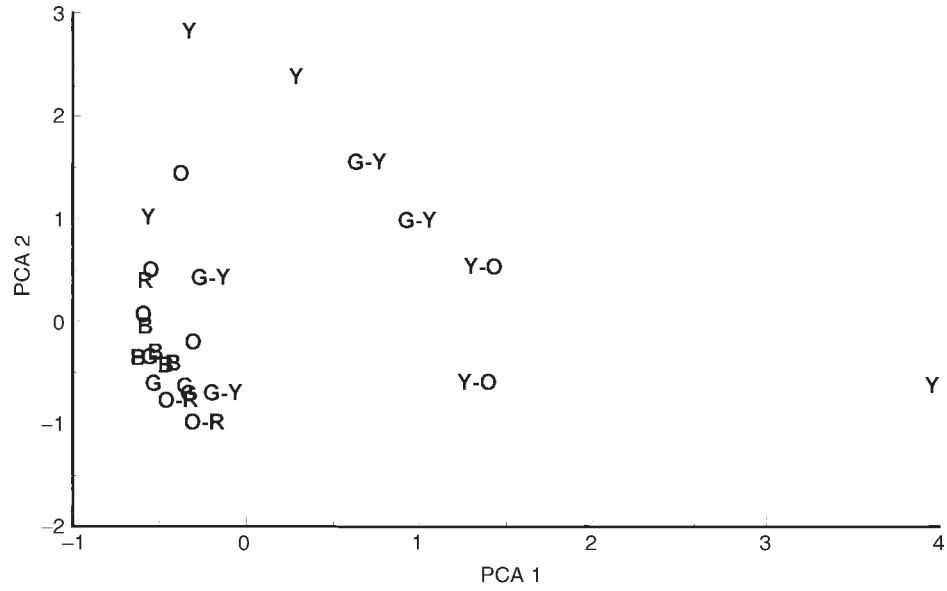

Fig. 8.2. Graph of PCA scores for the first two PCA axes, for the analysis using uncorrected fruit chemical and morphological traits (TIPS data). Factor loadings for the axes correspond to those listed in Table 8.3A. Symbols indicate ripe fruit colours of each species: R = red, B = black/purple, O = orange, Y = yellow, G = green.

Table 8.3. Results of principal component analysis. Data are factor loadings exceeding a value of 0.20 for the first two factors, as well as the per cent of total variation (%VAR) explained by each factor.

A. Fruit chemical and morphological traits (n = 26 species)

Factor	%VAR	RTGA	RPHN	RPRO	RSUG	UTGA	UPHN	UPRO	USUG	BLCK	GREN	YELL	ORNG	RED	WMS	PMS	SMS	PDM	DMP	DMS	SDM	ASM	SDN
TIPS		Factor loadings																					
1	28.98	0.54	0.41	–0.6	–0.35	0.40	0.40	–0.52	0.29	–0.38	–	0.82	–	–0.27	0.90	0.88	0.84	0.84	0.84	0.23	–	–	0.9
2	13.85	–0.50	–0.77	–	–	–0.73	–0.57	0.48	–	–	–	–0.20	0.34	0.29	0.33	0.28	0.42	0.47	0.37	–0.2	0.25	–0.31	0.24

B. Fruit chemical traits (n = 30 species)

Factor	%VAR	RTGA	RPHN	RPRO	RSUG	UTGA	UPHN	UPRO	USUG	GEND
TIPS		Factor loadings								
1	35.69	0.66	0.80	–	–0.62	0.70	0.68	–	–	n/a
2	21.15	0.22	0.31	–0.82	0.35	0.45	–	–0.84	0.51	n/a
IC										
1	35.30	0.87	0.92	–	–	0.85	0.39	–	0.28	n/a
2	30.15	0.22	–	0.92	–0.77	–0.41	–	0.83	–	n/a
PA										
1	36.16	0.67	0.81	–	–0.59	0.72	0.70	–	–	n/a
2	21.49	0.21	0.29	–0.82	0.40	0.43	–	–0.84	0.54	n/a
MAN										
1	20.91	0.66	0.82	–	–	0.86	–	–	–	–
2	16.65	–	–	0.71	0.57	–	–	0.80	–	–

C. Fruit morphological traits (n = 26 species)

Factor	%VAR	BLCK	GREN	YELL	ORNG	RED	WMS	PMS	SMS	PDM	DMP	DMS	SDM	ASM	SDN	GEND
TIPS		Factor loadings														
1	36.81	–0.28	–	–	–	–	0.95	0.90	0.96	0.95	–	–	0.96	–	0.74	n/a
2	17.61	–0.35	0.98	0.98	–0.41	–0.20	–	–	–	–	–	–	–	–0.24	–	n/a
IC																
1	36.01	–	–	–	–	–	0.97	0.94	0.96	0.89	–	–	0.96	–	0.70	n/a
2	19.87	–	–0.55	–	0.83	–	–	–	–	–	–0.75	0.91	–	–	–	n/a
PA																
1	38.72	–0.20	–	0.49	–	–	0.94	0.89	0.95	0.95	–	–	0.96	–	0.75	n/a
2	13.01	–0.22	–0.90	–0.42	0.62	0.27	–	–	–	–	0.31	–	–	–	–	n/a
MAN																
1	32.89	–	–	–	–	–	0.94	0.87	0.93	0.95	–	–	0.94	–	0.71	–
2	9.72	–0.55	–	–	0.41	0.62	–	–	–	–	0.37	–0.25	–	–0.26	–	0.57

Variable definitions:%VAR, percentage of total trait variation explained by each factor; RTGA, ripe-fruit TGA; RPHN, ripe-fruit total phenolics; RPRO, ripe-fruit total protein; RSUG, ripe-fruit total sugar; UTGA, unripe-fruit TGA; UPHN, unripe-fruit total phenolics; UPRO, unripe-fruit total protein; USUG, unripe-fruit total sugars; BLCK, ripe fruits dark blue/purple/black; GREN, ripe fruits green; YELL, ripe fruits yellow; ORNG, ripe fruits orange; RED, ripe fruits red; WMS, whole wet fruit mass (g); PMS, pulp wet mass (g); SMS, seed wet mass (g); PDM, pulp dry mass (g); DMP, dry-matter pulp; DMS, dry-matter seeds; SDM, seed dry mass (g); ASM, average seed dry mass (g); SDN, seed number per fruit; GEND, genetic distance. All chemical data are in mg g^{-1} dry mass.

Fruit chemistry

TIPS-based PCA of fruit chemical traits of 30 species (Fig. 8.3A; Table 8.3B) likewise shows a differentiation among species that may correspond to a gradient from mammal to avian dispersal. In this analysis, species are differentiated along the first PCA axis (35.69% of total variation) from fruits low in sugar and high in GAs and phenolics to those high in sugar and low in GAs and phenolics. As with regression analyses, PA-, IC- and MAN-based PCA analyses using the same data set suggest little effect of phylogeny (Fig. 8.3B; Table 8.3B). In all cases, species are distinguished primarily by fruit secondary chemistry (PCA axis 1) and secondarily by protein and sugar concentrations (PCA axis 2).

Fruit morphology

As for chemical traits, PCA analyses of morphological traits were relatively unaffected by phylogenetic correction (Fig. 8.3C, D; Table 8.3C). The results of these PCAs were consistent with those of the other PCAs; in this case, the difference is between species with black, orange and/or red fruits with low mass and low seed number and those with yellow or green fruits with high mass and high seed number. TIPS- and PA-based PCAs were most similar, whereas IC- and MAN-based PCAs differed from the other PCAs in not showing differentiation based upon fruit colour on the first axis, suggesting that fruit colour was somewhat related to phylogeny. This was supported by high factor loadings for genetic distance and for black, orange and red fruit colour on the second MAN-based PCA axis.

Conclusions and Significance of Our Study

Physiological and phylogenetic constraints

If secondary metabolites of ripe fruits serve some adaptive purposes, then our results should show substantial variation among species that is unexplained by phylogeny or by physiological constraints. Our preliminary analyses provide some evidence for physiological constraints on chemical traits of ripe fruits (i.e. some association between unripe- and ripe-fruit chemistry), but do not suggest that phylogeny has an important influence on fruit chemical or morphological trait variation within the species studied. Regarding the positive relationship between unripe- and ripe-fruit chemical characteristics, one might ask, 'Which is the chicken and which is the egg?' For example, is a positive relationship between GA content in ripe and unripe fruits more likely to be a consequence of the fruit's inability to remove all GAs during ripening of immature fruits (where the GAs presumably have some functional role) or a consequence of the need to build GA levels over a long growth period (perhaps coupled with functional roles in both fruit stages)? A constraint that 'accidentally' produces fruits toxic to dispersers would seem to be very costly in terms of fitness; meanwhile, some species (i.e. those with high TGA levels in unripe fruits and virtually none in ripe fruits) reduce TGA levels with apparent ease.

Our preliminary analyses also support the existence of independently evolved fruit-dispersal syndromes. Nevertheless, we cannot yet draw strong conclusions from these data because our phylogenetically corrected analyses are currently based on too few species (40 is generally considered a minimum (Martins, 2000)) and because phylogenetically corrected analyses based on fruit chemical traits were done separately from those using fruit morphological traits. We also cannot yet report phylogenetically corrected results examining the relationship of leaf chemistry to fruit chemistry (although our preliminary TIPS analysis suggest no relationship). In future work, we hope to assess all traits (genetic relatedness, leaf chemistry, fruit chemistry and fruit morphology) using a larger, single set of species. Related to the problem of low sample size is an incomplete sampling of fruit types and lack of replication at the lower levels of the phylogeny. As more (presumably similar) closely related species are added to the analysis, the strength of the phylogenetic effect may increase. Increased sampling within monophyletic clades will also strengthen our ability to estimate ancestral states, which is necessary for IC analysis.

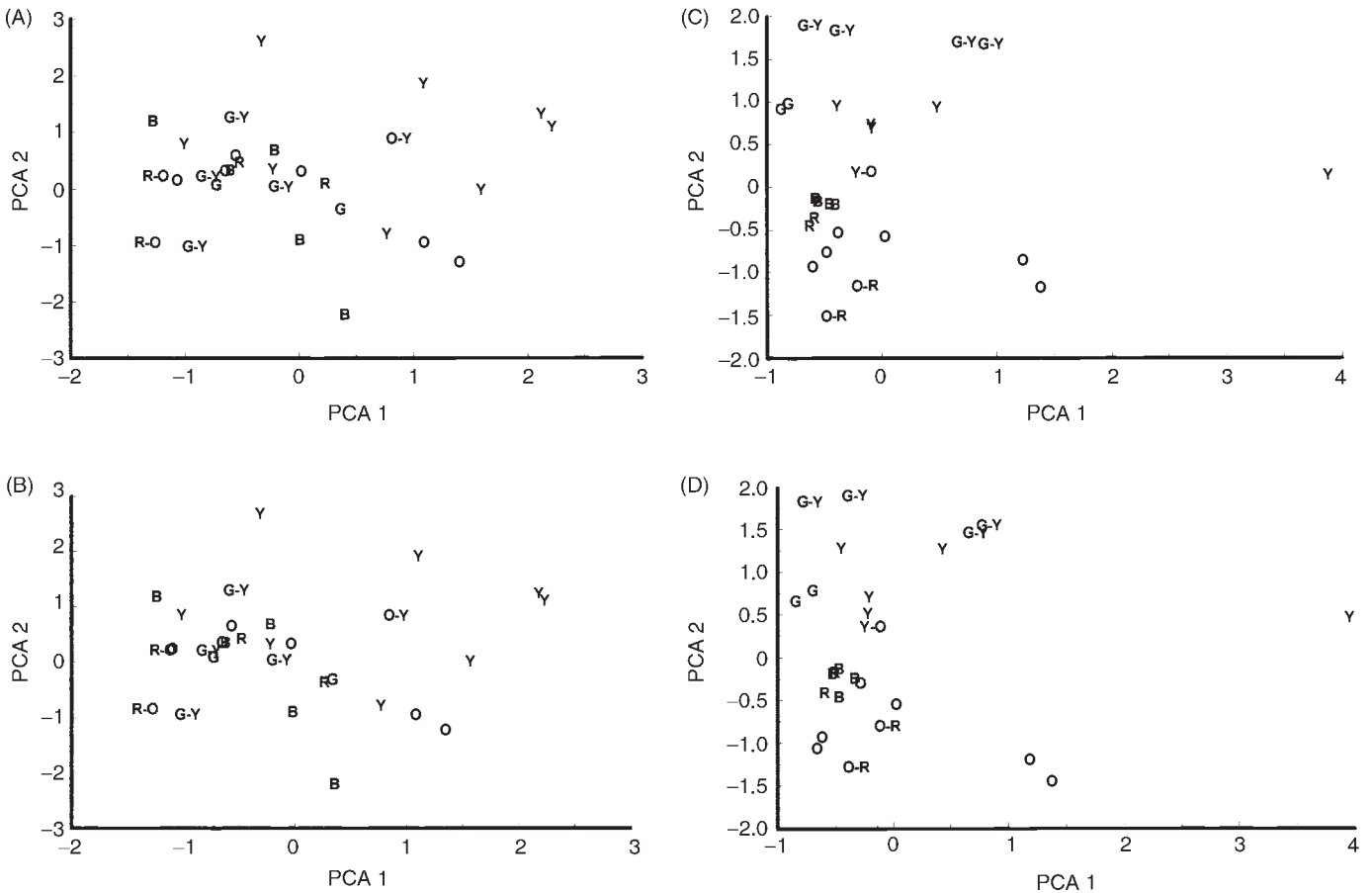

Fig. 8.3. Graphs of PCA scores for the first two PCAs using (A) uncorrected fruit chemical traits (TIPS data), (B) PA-corrected fruit chemical traits, (C) uncorrected fruit morphological traits (TIPS data), and (D) PA-corrected fruit morphological traits. Factor loadings correspond to those in Table 8.3B and C. Colour symbols correspond to those in Fig. 8.2.

Absent from our current analyses are estimates of variance due to within-species error and estimates of variance due to incorrect assumptions about the evolutionary model and/or incorrect specification of the phylogeny. So, in most cases (except MAN), we used standard parametric methods to determine the significance of statistical results. Martins and Hansen (1997) and Garland and Ives (2000) describe general approaches to incorporating within-species and phylogenetic sources of error into such analyses, which we plan to incorporate into our final analyses. Because our current analyses show little effect of phylogeny, we can only say that results for all methods of phylogenetic correction were basically similar.

'Phylogeny vs. ecology' vs. 'phylogeny and ecology'

Our study is focused mainly on the issue of phylogenetic effects and yet the distinction between ecology and phylogeny is not all that clear. In fact, some ecologists (e.g. Westoby *et al.*, 1995a,b,c) argue that trait variation across taxa is so inextricably intertwined with history that 'phylogeny' cannot be separated from 'ecology'. This is because related species may inherit similar habitats and selective regimes from their ancestors (the 'phylogenetic niche conservation' of Harvey and Pagel (1991)). Our inclination is that the IC and MAN methods might be more acceptable than PA, because variation attributed to phylogeny is not entirely removed from consideration when using IC or MAN. IC has lately become the method of choice for studies of plant evolution (Silvertown and Dodd, 1997), and progress has been made in reconciling the PA and IC methods into a general approach (see Martins and Hansen, 1997). On the other hand, since ancestral states must be estimated for IC, this method seems to require exhaustive sampling within clades to ensure valid ancestral state reconstructions. Considering all factors, the MAN technique appears to be a very promising approach to phylogenetic correction.

Westoby *et al.* (1995 a,b,c) and Givnish (1997) argue that all statistical 'phylogenetically correct' methods can be biased under certain circumstances: i.e. when the evolutionary model is incorrect (as in the case of strong directional selection), or when a trait or combination of traits of interest arises only a few times and it is assumed a priori that their persistence within a clade is a 'constraint'. In the latter case, the association of a trait (e.g. high ripe-fruit GA content) with an ecological correlate (e.g. dispersal by mammals) may seem to be explained by phylogeny and yet may have been adaptive when it first appeared and could have been maintained by selection thereafter. It may be equally plausible for correlations among traits to be maintained within clades by selective forces as it is for them to result from time-lags or from genetic, physiological and/or developmental constraints. Thus, neither adaptive nor non-adaptive explanations are necessarily more parsimonious. Adaptive explanations for traits and for covariation among traits are thus warranted in the absence of direct evidence for mechanisms of constraint, coupled with the presence of evidence of their current functions and/or fitness effects. This is a difficult issue to address and bolsters our belief that examinations of the functions and fitness values of fruit traits must remain an important approach to determining adaptive significance.

Future Work

How variable are closely related plant species in ripe-fruit secondary chemistry, and are differences among species the result of selection pressures related to frugivory? The rationale and study design needed to answer this question depend strongly on the results of a phylogenetically based study. The general approach is to study frugivory in the field, focusing on plants identified as key species for comparison. If highly contrasting taxa are common within clades (weak phylogenetic effects), one might ask questions concerning differences in the dispersers of these species. Associations of certain frugivore types with certain fruit chemical types would provide the strongest evidence of coadaptation. If one concludes, on the other hand, that variation in fruit chemical traits primarily reflects variation among clades (strong phylogenetic effects), one might question whether contrasting clades

differ in the dispersers associated with each. One might find that species within clades are, indeed, dispersed by similar disperser types (e.g. some clades by passerine birds, others by bats). In either case, extant frugivore–fruit-type associations could be interpreted either as 'old' coadaptive relationships or as the result of similar extant frugivores selecting related fruit species because of phylogenetically constrained physiological and ecological similarities – the 'ecological fitting' of Janzen (1985). Regardless, evidence would exist for the importance of secondary chemistry in explaining current fruit-use patterns.

The general approach described here differs from that taken in most comparative studies of fruit traits, which tend to focus on the traits of many species occupying one habitat whose fruit vary strongly in secondary chemistry. Although more difficult to conduct, examination of evolutionary divergence within a group of phylogenetically related species occupying different habitats or selective regimes could be more revealing than studies focusing on such habitat-defined species assemblages, and analyses of fruit secondary chemical profiles are technically facilitated. Regardless, good estimates of phylogenetic relationships are essential for our approach.

We recommend continued exploration of the ecological functions of ripe-fruit secondary metabolites as a means of better understanding possible consequences for frugivory, seed dispersal and plant fitness. Regardless of conclusions about the evolution of fruit traits, a focus on secondary metabolites should continue to enlighten the understanding of the ecology of fruit–frugivore interactions. In addition to their importance for addressing evolutionary and ecological questions, studies of fruit secondary metabolites have significance for medicinal phytochemistry and for conservation. Using our study as an example, fruit GAs are of potential use as: precursors for steroid synthesis, anticancer agents, fungicides, molluscicides, pesticides, herbicides, antiparasitic agents, neurologically active agents and cholesterol-lowering agents (Cipollini, 2000). Focused study of such chemicals could result in the identification of new sources of known compounds or sources of novel compounds. This possibility provides an important argument for conservation efforts directed towards such taxa of potential medical importance (Tewksbury *et al.*, 1999).

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References

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, D.F. and Donoghue, M.J. (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82, 247–277.

Birner, J. (1969) Determination of total steroid bases in *Solanum* species. *Journal of Pharmaceutical Science* 58, 258–259.

Böhning-Gaese, K. and Oberrath, R. (1999) Phylogenetic effects on morphological, life-history, behavioural, and ecological traits of birds. *Evolutionary Ecology Research* 1, 347–364.

Böhning-Gaese, K., Halbe, B., Lemoine, N. and Oberrath, R. (2000) Factors influencing the clutch size, number of broods and annual fecundity of North American and European land birds. *Evolutionary Ecology Research* 2, 823–839.

Bohs, L. (2000) Slicing up the Solanums: major lineages and morphological synapomorphies. *American Journal of Botany* 87(6), 115 (Abstract).

Bohs, L. and Olmstead, R.G. (1997) Phylogenetic relationships in *Solanum* (*Solanaceae*) based on ndhF sequences. *Systematic Botany* 22, 5–17.

Bohs, L. and Olmstead, R.G. (1999) *Solanum* phylogeny inferred from chloroplast DNA sequence data. In: Nee, M., Symon, D.E., Lester, R.N. and Jessop, J.P. (eds) *Solanaceae IV: Advances in Biology and Utilization*. Royal Botanic Gardens, Kew, UK, pp. 97–110.

Bohs, L. and Olmstead, R.G. (2001) A reassessment of *Normania* and *Triguera* (*Solanaceae*). *Plant Systematics and Evolution* (in press).

Bradley, V., Collins, D.J., Eastwood, F.W., Irvine, M.C. and Swan, J.M. (1979) Distribution of steroidal alkaloid in Australian species of *Solanum*. In: Hawkes, J.G., Lester, R.N. and Skelding, A.D. (eds) *The Biology and Taxonomy of the Solanaceae*. Academic Press, London, UK, pp. 203–209.

Bremer, B. and Eriksson, O. (1992) Evolution of fruit characters and dispersal modes in the tropical family *Rubiaceae*. *Biological Journal of the Linnean Society* 47, 79–95.

Budini, R., Tonelli, D. and Girotti, S. (1980) Analysis of total phenols using the Prussian blue method. *Journal of Agricultural and Food Chemistry* 28, 1236–1238.

Cheverud, J.M., Dow, M.M. and Leutenegger, W. (1985) The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weights among primates. *Evolution* 39, 1335–1351.

Cipollini, M.L. (2000) Secondary compounds in fleshy fruits: evidence for adaptive functions. *Revista Chilena de Historia Natural* 73, 243–252.

Cipollini, M.L. and Levey, D.J. (1997a) Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78, 782–798.

Cipollini, M.L. and Levey, D.J. (1997b) Antifungal activity of *Solanum* fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology* 78, 799–809.

Cipollini, M.L. and Levey, D.J. (1997c) Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. *American Naturalist* 150, 346–372.

Cipollini, M.L. and Levey, D.J. (1998) Secondary metabolites as traits of ripe fleshy fruits: a response to Eriksson and Ehrlen. *American Naturalist* 152, 908–911.

Courtenay, O. (1994) Conservation of the maned wolf: fruitful relationships in a changing environment. *Canid News* 2, 1–5.

D'Arcy, W.G. (1972) Solanaceae studies II: typification of subdivisions of *Solanum*. *Annals of the Missouri Botanical Garden* 59, 262–278.

D'Arcy, W.G. (1991) The *Solanaceae* since 1976, with a review of its biogeography. In: Hawkes, J.G., Lester, R.N. and Skelding, A.D. (eds) *Solanaceae III: Taxonomy, Chemistry, Evolution*. Royal Botanic Gardens, Kew, UK, pp. 75–137.

Debussche, M. and Isenmann, P. (1989) Fleshy fruit characters and the choices of bird and mammal seed-dispersers in a Mediterranean region. *Oikos* 56, 327–338.

de Queiroz, K. (1996) Including characters of interest during tree reconstruction and the problems of circularity and bias in studies of character evolution. *American Naturalist* 148, 700–708.

Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19, 11–15.

Ehrlen, J. and Eriksson, O. (1993) Toxicity in fleshy fruits – a non-adaptive trait? *Oikos* 66, 107–113.

Eriksson, O. and Ehrlen, J. (1998) Secondary metabolites in fleshy fruits: are adaptive explanations needed? *American Naturalist* 152, 905–907.

Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist* 125, 1–15.

Garland, T., Jr and Ives, A.R. (2000) Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist* 155, 346–364.

Gautier-Hion, A. (1990) Interactions among fruit and vertebrate fruit-eaters in an African tropical rain forest. In: Bawa, K. and Hadley, M. (eds) *Reproductive Ecology of Tropical Forest Plants*. Man and the Biosphere Series, Vol. 7, Parthenon Press, Paris, France, pp. 219–232.

Gittleman, J.L. and Kot, M. (1990) Adaptation: statistics and a null model for estimating phylogenetic effects. *Systematic Zoology* 39, 227–241.

Givnish, T.J. (1997) Adaptive radiation and molecular systematics: issues and approaches. In: Givnish, T. and Sytsma, K. (eds) *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, Cambridge, UK, pp. 1–54.

Harvey, P.H. and Pagel, M.D. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford, UK.

Herrera, C.M. (1992) Interspecific variation in fruit shape: allometry, phylogeny, and adaptation to dispersal agents. *Ecology* 73, 1832–1841.

Herrera, C.M. (1998) Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: a 12-year study. *Ecological Monographs* 68, 511–538.

Howe, H.F. (1984) Constraints on the evolution of mutualisms. *American Naturalist* 123, 764–777.

Janson, C.H. (1983) Adaptation of fruit morphology to dispersal agents in a neotropical forest. *Science* 219, 187–188.

Janzen, D.H. (1985) On ecological fitting. *Oikos* 45, 308–310.

Jones, C.G., Hare, J.D. and Compton, S.J. (1989) Measuring plant protein with the Bradford assay. 1. Evaluation and standard methodology. *Journal of Chemical Ecology* 15, 979–992.

Jordano, P. (1987a) Diet, fruit choice and variation in body condition of frugivorous warblers in Mediterranean scrubland. *Ardea* 76, 193–209.

Jordano, P. (1987b) Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *American Naturalist* 129, 657–677.

Jordano, P. (1995) Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant–animal interactions. *American Naturalist* 145, 163–191.

Kingsbury, J.M. (1964) *Poisonous Plants of the United States and Canada*. Prentice-Hall, Englewood Cliffs, New Jersey.

Legendre, P., Lapointe, F.-J. and Casgrain, P. (1994) Modeling brain evolution from behaviour: a permutational regression approach. *Evolution* 48, 1487–1499.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27, 209–220.

Martins, E.P. (2000) *COMPARE: Computer Programs for the Statistical Analysis of Comparative Data*, Version 4.3. Department of Biology, University of Oregon, Eugene, Oregon. http://darkwing.uoregon.edu/~compare4/

Martins, E.P. and Hansen, T.F. (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist* 149, 646–667.

Nee, M. (1991) 17. Synopsis of *Solanum* Section Acanthophora: a group of interest for glycoalkaloids. In: Hawkes, J.G., Lester, R.N., Nee, M. and Estrada, J. (eds) *Solanaceae III: Taxonomy, Chemistry, Evolution*. Royal Botanical Gardens Kew and Linnean Society of London, London, pp. 257–266.

Olmstead, R.G. and Palmer, J.D. (1997) Implications for the phylogeny, classification, and biogeography of *Solanum* from cpDNA restriction site variation. *Systematic Botany* 22, 19–29.

Olmstead, R.G., Sweere, J.A., Spangler, R.E., Bohs, L. and Palmer, J.D. (1999) Phylogeny and provisional classification of the *Solanaceae* based on chloroplast DNA. In: Nee, M., Symon, D.E., Lester, R.N. and Jessop, J.P. (eds) *Solanaceae IV: Advances in Biology and Utilization*. Royal Botanic Gardens, Kew, pp. 111–137.

Ripperger, H. and Schreiber, K. (1981) Solanum steroid alkaloids. In: Manske, R.H.F. and Rodrigo, R.G.A. (eds) *The Alkaloids: Chemistry and Physiology*, Vol. XIX. Academic Press, New York, pp. 81–192.

Schreiber, K. (1963) Isolierung von Solasodinglykosiden aus Pflanzen der Gattung *Solanum* L. *Solanum*-Alkaloide. XXVIII. *Mitteilung. Kulturpflanze* 11, 451–501.

Schreiber, K. (1968) Steroid alkaloids: the *Solanum* group. In: Manske, R.H.F. (ed.) *The Alkaloids, Chemistry and Physiology*, Vol. X. Academic Press, New York, pp. 1–192.

Silvertown, J. and Dodd, M. (1997) Comparing plants and connecting traits. In: Silvertown, J., Franco, M. and Harper, J.L. (eds) *Plant Life Histories: Ecology, Phylogeny and Evolution*. Cambridge University Press, Cambridge, UK, pp. 3–35.

Smith, D. (1981) *Removing and Analyzing Total Nonstructural Carbohydrates from Plant Tissue*. Publication R2107, Wisconsin University Extension Service, Madison, Wisconsin, USA.

Smouse, P.E., Long, J.C. and Sokal, R.R. (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* 35, 627–632.

Spooner, D.M., Olmstead, R.G. and Bohs, L. (1999) Current data on the systematics of the *Solanaceae*, with a focus on potatoes and tomatoes. In: *Plant and Animal Genome VII. Abstracts*, San Diego, CA. p.67.

SPSS, Inc. (1999) *SPSS for Windows*, Version 10.0. SPSS, Inc., Chicago, Illinois.

Stiles, F.G. and Rosselli, L. (1992) Consumption of fruits of the *Melastomataceae* by birds: how diffuse is coevolution? *Vegetatio* 107, 57–74.

Swofford, D. (2000) *PAUP* 4.0*. Beta version 4.0b3a. Sinauer Associates, Sunderland, Massachusetts.

Tewksbury, J.J., Nabhan, G.P., Norman, D., Suzan, H., Tuxhill, J. and Donovan, J. (1999) 'In situ' conservation of wild chiles and their biotic associates. *Conservation Biology* 13, 98–107.

van der Pijl, L. (1969) *Principles of Dispersal in Higher Plants*. Springer-Verlag, Berlin, Germany.

van Gelder, W.M. (1990) Chemistry, toxicology, and occurrence of steroidal glycoalkaloids: potential contaminants of the potato (*Solanum tuberosum* L.). In: Rizk, A.-F.M. (ed.) *Poisonous Plant Contamination of Edible Plants*. CRC Press, Boca Raton, Florida, pp. 117–156.

Westoby, M., Leishman, M. and Lord, J. (1995a) On misinterpreting the phylogenetic correction. *Journal of Ecology* 83, 531–534.

Westoby, M., Leishman, M. and Lord, J. (1995b) Further remarks on phylogenetic correction. *Journal of Ecology* 83, 727–729.

Westoby, M., Leishman, M. and Lord, J. (1995c) Issues of interpretation after relating

comparative datasets to phylogeny. *Journal of Ecology* 83, 892–893.

Wheelwright, N.T. (1985) Fruit size, gape width, and the diets of fruit-eating birds. *Ecology* 66, 808–818.

Willson, M.F., Irvine, A.K. and Walsh, N.G. (1989) Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographic comparisons. *Biotropica* 21, 133–147.