



A survey of seed and seedling characters in 1744 Australian dicotyledon species: cross-species trait correlations and correlated trait-shifts within evolutionary lineages

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Seedling traits have been described across 1744 species of Australian plants. Six traits were coded as binary alternatives: (1) phanerocotily vs cryptocotily; (2) first leaf scale-like vs leaf-like; (3) first leaf single vs paired; (4) cotyledons hairy vs. glabrous; (5) hypocotyl hairy vs glabrous, and (6) embryo green vs non-green at maturity. Seed volume was calculated from measurements of seed dimensions. Three approaches were used in analysing the data: (1) the taxonomic distribution of binary character states was described; (2) the strength of bivariate character associations was quantified at species level ('cross-species' correlations and regressions); (3) the data were arrayed on a phylogenetic tree in order to analyse by 'phylogenetic regression' for correlated evolutionary shifts in trait pairs. All the traits appeared evolutionarily malleable. For example, while cryptocotily was the minority condition (22% of species), it occurred in many different taxa (124 genera, 40 families, 24 orders) with high levels of polymorphy (16%, 25% and 42% for genera, families and orders). Similarly, the less common attribute states for first leaf type, hypocotyl texture, cotyledon texture and embryo colour occurred in species from right across the dicotyledon phylogeny, as did independent evolutionary divergences in each of these characters. These patterns indicate that debate over which conditions are primitive and which are advanced will not have any general answer, only an answer for a specific branch-step in the phylogenetic tree. In nearly all cases, correlated-divergence analyses showed the same patterns as cross-species analyses. The strongest associations were between seed volume and cryptocotily, seed volume and scale-like first leaf, and seed volume and presence of green embryo. In addition, cryptocotily and scale-like first leaf, and cotyledon and hypocotyl type, were strongly associated in both correlated-divergence and cross-species analyses. Interpretation of results was mostly presented with respect to seed size, a trait which we consider to be pivotal in a species' seedling establishment strategy. All possible pairwise combinations of binary seedling traits were found in our study species. Taken together, the various lines of evidence presented here suggest that the traits have assorted more or less independently of each other and provide no evidence of functional groups based on these attributes. Thus, the several existing seedling typologies should be regarded as classifications of convenience rather than as reflecting fundamental types.

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INTRODUCTION

As a part-time activity across 40 years, one of us (H.T.C.) has noted the seedling traits of Australian dicots. These notes have now accumulated to a dataset covering 1744 species, with the majority derived from seeds germinated on a windowsill and the remainder taken from the literature (Clifford, 1987; Hyland & Whiffin 1993). The present paper examines what constellations of traits are most commonly found together in seedlings among these species, that is, correlations in trait distribution across present-day species. Further, the data analysis arrays the species on the phylogenetic tree of dicots and considers patterns in the correlated divergence of traits, as a complement to analysing correlation across present-day species.

Previous seedling character surveys and classification schemes fall into three broad categories: (1) taxonomic (facilitating identification of seedlings found in the field; e.g. Duke, 1969); (2) ecological (delineation of morphological or functional types; e.g. Ng, 1978; de Vogel, 1980; Hladik & Miquel, 1990; Garwood, 1995); and (3) evolutionary (designation of character states as ancestral or derived; e.g. Clifford, 1984, 1987, 1991; Yakovlev & Zhukova 1980; Dahlgren, 1980; Burt, 1991). Surveys of the first two types have been largely restricted to seedlings of tropical floras. Probably the most surveyed seedling character is that of cotyledon position and degree of exposure at time of germination (dating back to late last century; see de Vogel, 1980). Cotyledons below the soil surface are ‘hypogeal’ while those raised above the soil by extension of the hypocotyl are ‘epigeal’. Duke (1965) emphasized the degree of cotyledon exposure, coining the terms ‘cryptocotyly’ (for the condition in which the cotyledons remain enveloped in the persistent fruit wall and/or testa, together with which they are shed) and ‘phanerocotyly’ (for the condition in which the cotyledons become entirely exposed, free from fruit wall and testa after germination). While most epigeal seedlings are phanerocotylar and hypogeal seedlings cryptocotylar, the terms are not synonymous. For example, *Lobelia* has hypogeal,

phanerocotylar germination and *Alyxia ruscifolia* is cryptocotylar yet epigeal (Clifford, 1991).

Along with cotyledon position and exposure, cotyledon functional morphology (i.e. the spectrum from globoid, food-storing, non-photosynthetic cotyledons to foliaceous photosynthetic cotyledons) has been used in classification of seedlings into functional types (Kitajima, 1995; Garwood, 1995). Functional classifications based on cotyledon characters only, while undoubtedly useful, are rather limited since other plant parts must also play vital roles in early seedling establishment (Garwood, 1995). Of course, many characters can potentially be measured. We present data on a moderate number of easily measured seed and seedling characters which have been surveyed before, or suggested as being useful, for taxonomic and/or functional seedling classification (e.g. de Vogel, 1980). Characters included germination mode, morphology and arrangement of the first leaves, presence of hairs on hypocotyl and cotyledons, presence of chlorophyll in the embryo, and seed size.

METHODS

Data set

Seeds of 1679 indigenous Australian dicotyledon species and 65 dicotyledon weed species were germinated in petri dishes on filter paper resting on a moisture reservoir of damp soil or sand. Dishes were placed near the window in the office of H.T.C. If germination did not occur, various treatments were applied such as increased temperature, vernalisation in a standard refrigerator, fluctuating temperature, and scarification and leaching of seeds or fruits in running water. Seven seed and seedling characters were scored in total, although the number of empty cells varied between characters. Six of the seven were binary qualitative characters: germination mode (1738 spp) phanerocotylar 1, cryptocotylar 2; first leaf (940 spp) leaf-like 1, scale-like (cataphyll) 2; first node (840 spp) leaf single 1, leaves paired 2; hypocotyl (567 spp) glabrous 1, hairy 2; cotyledons (586 spp) glabrous 1, hairy 2; embryo (296 spp) colourless 1, green 2. With the exception of first node type, coding was such that 1 was the more common state at species level. Seeds were assumed to be ellipsoids and the volumes calculated for 302 species from measurements of seed length (l), breadth (b) and width (w) (volume = $\pi/6.l.b.w$). Seed volume ranged from 0.001 mm³ (*Drosera burmanii*) to 93 530 mm³ (*Aleurites molucana*) and was log transformed prior to analyses since the distribution was log normal.

In addition to the seven primary characters, fruit type was recorded for all species for which we had seed volume and/or embryo data (total 488 species). Nine categories were recorded: achene (10 species), berry (79), capsule (158), cypsela (18, all Asteraceae), drupe (121; 88 of which from Lauraceae), legume (69, all Fabales), nut (16), schizocarp (9), and utricle (8, all Caryophyllales). Data on presence/absence of endosperm (Clifford & Ludlow, 1978) and average seed oil content (Earle & Jones, 1962; Jones & Earle, 1966; Barclay & Earle, 1974; Levin, 1974) were collated in order to explore relationships suggested by the results and/or relevant literature.

Analyses

Three approaches were used in analysing the data. Firstly, the taxonomic distribution of binary character states was described. Classification of species into

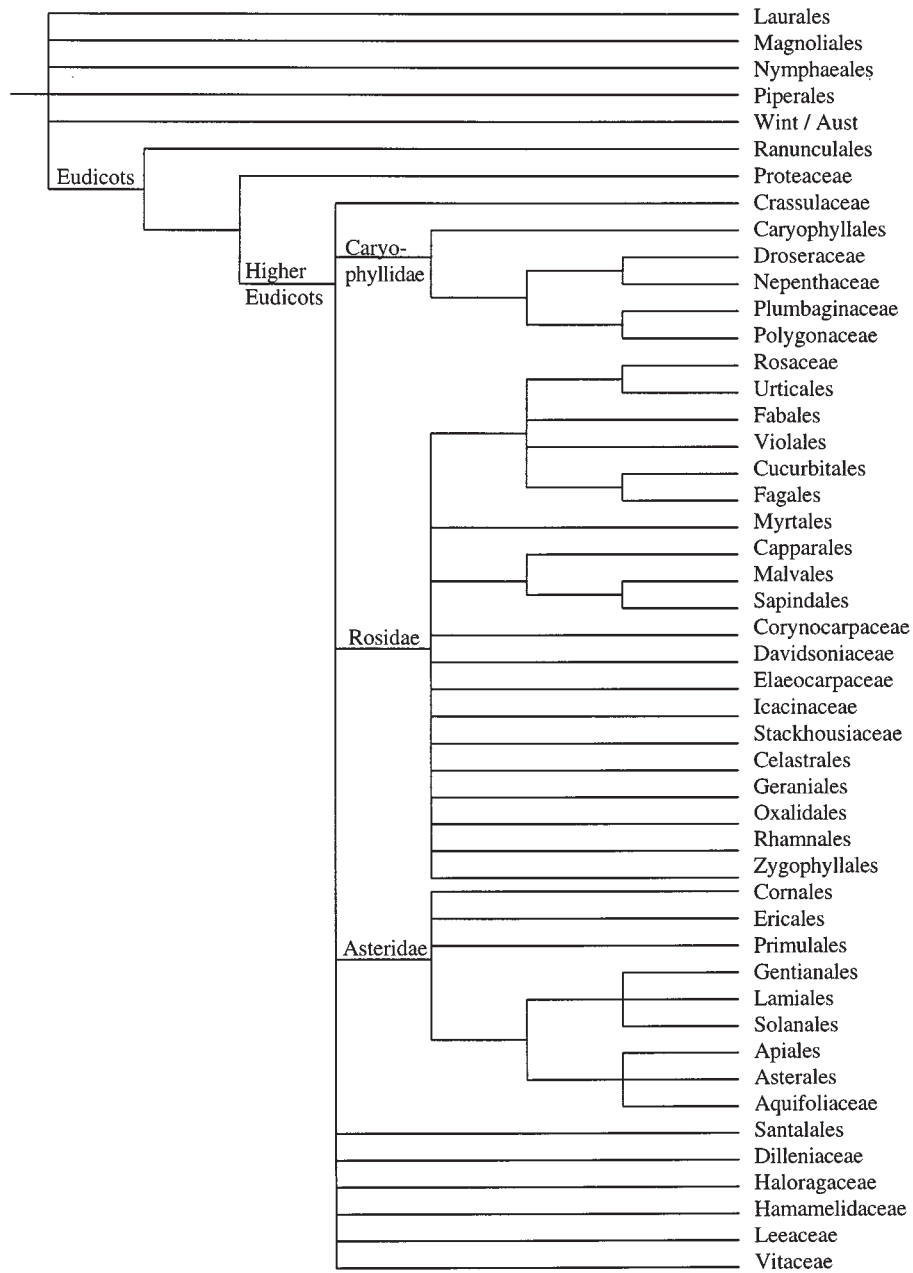


Figure 1. Higher-level phylogenetic tree adapted from the Uppsala phylogeny. Families in each order are listed in Appendix 1. Wint/Aust: Winteraceae + Austrobaileyaceae.

families and higher groupings followed Bremer's 'Uppsala Phylogeny' (Fig. 1) (<http://www.systbot.uu.se/classification/overview.html>; as at September 1996), while generic delineation followed Harden (1990) and *Flora of Australia* (1981–). Secondly, the

strength of bivariate character associations was quantified at species level ('cross-species' correlations and regressions; SPSS for Windows v. 8.0). Thirdly, phylogenetic information was incorporated into analyses using a technique known as phylogenetic regression (Grafen, 1989, 1992; described below) in order to identify common correlated character shifts within evolutionary lineages.

Cross-species and phylogenetic analyses address different questions and should be thought of as complementary (Price, 1997; Westoby *et al.*, 1998). For example, if two traits are correlated in a data set of randomly selected species but only due to strong correlations within a small number of speciose clades, it is correct to conclude that the traits are generally correlated but it is not true that the possible causal link has occurred in many evolutionary lineages. Note that this neither verifies the presence or lack of a causal link between the attributes. That related species share many attributes is obvious. While this fact is sometimes attributed to a nebulous concept known as 'phylogenetic constraint' (e.g. Kelly & Purvis, 1993), we prefer to attribute it to 'phylogenetic niche conservatism' (Lord, Westoby & Leishman, 1995), i.e. closely related species tend to be similar because they share a common ancestor and because they are subject to similar continuing forces of natural selection. If one's interest is in identifying correlated attribute changes which have occurred repeatedly in different lineages, then individual evolutionary divergences (rather than individual species) should form the data points on which appropriate statistical tests are made. This idea is known as the 'radiation principle' (Ridley, 1983).

Phylogenetic regression is one of several methods which make use of this principle. It has the advantage that it is appropriate for continuous and discrete data, and provides the hypothesis testing facilities of general linear models (Grafen, 1992; Grafen & Ridley, 1996). The method requires the drawing and coding of a 'working phylogeny', the best supported hypothesis concerning the evolution of the species, containing only monophyletic groups. Polychotomies are used to represent information that is unknown or doubtful. The data set of character values for extant species undergoes a transformation called 'hanging on the tree', whereby each higher node acquires an average value based on the value of nodes directly below it (a weighted average if the path segments have different lengths). These values are interpreted in relation to the set of descendant species (each node is next re-expressed as a difference from its parent node) rather than interpreted as reconstructions of ancestral character states. The method regresses the values of any two characters (controlling for any number of additional variables) on each other within each radiation in the phylogeny and combines the regressions into the 'phylogenetic regression'. Each extra node in the phylogenetic tree adds an additional degree of freedom to the analysis; conversely, where no change has occurred in the variable between successive nodes, a degree of freedom is lost.

A phylogenetic tree linking the 1744 species was constructed from taxonomic information. Where resolution was poor, additional phylogenetic information was taken from consensus cladistic trees in recent publications utilizing either molecular or morphological data (details available from authors). For Myrtaceae, taxonomic studies were in progress but not published and the relevant taxonomists were contacted and asked to improve the resolution and accuracy of our phylogeny, where possible. Since the affinity of a number of families was uncertain at order level, these taxa were treated as monofamilial clades (or bifamilial in the case of Austrobaileyaceae + Winteraceae) and placed in the tree as basal polychotomies at the lowest node where affinity was certain. This is the fairest manner of

incorporating such groups into a phylogenetic analysis such as this since each extra node gives an extra potential degree of freedom. Placing a taxon in a likely-to-be incorrect position is undesirable where this gives an extra degree of freedom, but placing the taxon in a polychotomy assumes less knowledge and has no systematic deleterious effect on the analysis (i.e. it does not bias the analysis). For example, Dilleniaceae, Haloragaceae, Hamamelidaceae, Leeaceae and Vitaceae are 'Higher Eudicots' in Bremer's system but do not fit in the Caryophyllidae, Rosidae or Asteridae. Consequently, each of these families was assigned its own branch from the divergence which gave rise to the Caryophyllidae, Rosidae and Asteridae. Similarly, Corynocarpaceae, Davidsoniaceae, Elaeocarpaceae, Icacinaceae and Stackhousiaceae are known to be Rosids but their affinity with the known clades within Rosidae is uncertain. Thus they were placed as basal branches within this group. Treating families of uncertain affinity in this way increased the number of 'orders' sampled and particularly the number of orders with few species in them. This may have affected the proportion of attribute states at order level, but it did not necessarily bias the results in any direction. The creation of these additional orders has no effect on phylogenetic regression since no extra divergences have been added.

In the creation of weighted averages for parent nodes, path segment lengths are taken into account. Although taxonomic levels are arbitrary in many respects, node heights were fixed such that genera, families, orders, superorders etc were equally spaced and at the same heights in all lineages (using the 'fully general method' of Grafen, 1989). Where additional divergence information was known in between these levels, additional nodes were spaced equally apart in height. A second set of analyses were carried out using the default 'figure 2' method (Grafen, 1989), in which the height of each node is initially set in proportion to the number of daughter nodes stemming from that node, then path lengths are distorted by a scaling factor, ρ , which is estimated by maximum likelihood simultaneously with the regression parameters. However, only results from the first set of analyses are presented since they were virtually identical to those from the 'figure 2' method, and we feel that this is a more correct method of creating a phylogenetic tree when not all species in each taxon have been sampled.

RESULTS

Taxonomic distribution of binary character states

Germination type

In this section numbers given in brackets refer to the number of species sampled for the particular attribute under discussion rather than the total number of species in that taxon in the data set. Character states for families and orders are given in Appendix 1.

Of the 1738 species sampled for this character, 388 (22%) had cryptocotylar germination (Table 1). These species were widely distributed taxonomically, coming from 124 genera, 40 families and 24 orders. Sixty-one per cent of cryptocotylar species were from Rosidae and 24% from Laurales, these groups contributing 63% and 6% of the 1744 species, respectively (i.e. Laurales was 'over-represented' in this respect). The proportion of taxa exclusively cryptocotylar dropped to only 3–5% at

TABLE 1. Binary character state proportions by taxonomic level. 1, 2: proportion exclusively exhibiting state 1 or 2; 1&2: proportion polymorphic for that character; *n*: number of taxa.

character	level	1	2	1&2	<i>n</i>
germination	species	0.78	0.22		1738
<i>phanerocotylar 1</i>	genus	0.81	0.03	←→ 0.16	637
<i>cryptocotylar 2</i>	family	0.70	0.05	0.25	128
	order	0.54	0.04	0.42	48
first leaf	species	0.78	0.22		940
<i>leaf-like 1</i>	genus	0.84	0.02	←→ 0.14	359
<i>scale-like 2</i>	family	0.75	0.07	0.19	102
	order	0.57	0.02	0.40	42
first node	species	0.41	0.59		840
<i>leaf single 1</i>	genus	0.48	0.05	←→ 0.46	351
<i>paired 2</i>	family	0.43	0.30	0.27	103
	order	0.43	0.05	0.52	42
hypocotyl	species	0.78	0.22		567
<i>glabrous 1</i>	genus	0.71	0.05	←→ 0.24	309
<i>hairy 2</i>	family	0.50	0.20	0.30	92
	order	0.33	0.08	0.60	40
cotyledons	species	0.84	0.16		586
<i>glabrous 1</i>	genus	0.80	0.04	←→ 0.16	319
<i>hairy 2</i>	family	0.55	0.15	0.30	97
	order	0.41	0.07	0.51	41
embryo	species	0.84	0.16		296
<i>colourless 1</i>	genus	0.76	0.02	←→ 0.22	156
<i>green 2</i>	family	0.59	0.20	0.21	51
	order	0.52	0.07	0.41	27

higher taxonomic levels, with the percentage of polymorphic taxa increasing steadily from 16% at genus level to 42% at order level. With the exception of Lauraceae (91 species; Laurales) and Clusiaceae (13 species; Violales), all exclusively-cryptocotylar orders or families (Appendix 1) had only one species sampled for this character. Well represented exclusively-phanerocotylar orders (e.g. orders with more than 10 species sampled) included Capparales (11 species), Caryophyllales (40), Fagales (14), Oxalidales (20) and Solanales (29). Of the 90 families containing phanerocotylar species only, 30 had only 1 species while 9 had more than 10 species: Elaeocarpaceae (affinity uncertain at order level; hereafter, such taxa assigned 'a.u.'), Rubiaceae (Gentianales, 24), Convolvulaceae (Solanales, 22), Chenopodiaceae (Caryophyllales, 18), Monimiaceae (Laurales, 16), Cunoniaceae (Oxalidales, 15), Pittosporaceae (Apiales, 14), Casuarinaceae (Fagales, 11), Vitaceae (a.u., 11).

First leaf type

Twenty-two per cent of species (213 of 940) had scale-like first leaves or 'cataphylls'. These species came from 58 genera, 26 families and 18 orders. Forty-nine per cent of the cataphyllous species were from Rosidae and 42% from Lauraceae. The proportion of taxa exclusively cataphyllous dropped to only 2% at order level, with the percentage of polymorphic taxa increasing from 14% at genus level, to 19% of families and 40% of orders. No orders and only one family were exclusively cataphyllous and well represented (Casuarinaceae; 10 species; Fagales). Well represented orders and families containing species with leaf-like first leaves only included Apiales (20 species), Lamiales (24), Violales (21), Asteraceae (Asterales, 18), Euphorbiaceae (Violales, 18), Rutaceae (Sapindales, 17), and Apiaceae (Apiales, 11).

First node type

Fifty-nine per cent of species (496 of 840) had paired first leaves. Five per cent of genera, 30% of families and 5% of orders exhibited paired first leaves exclusively, while the proportion with single first leaves-only ranged between 41% and 48% at all taxonomic levels. Thus % polymorphy showed approximately the opposite trend to % paired first leaves (the proportion dropped from 46% of genera to 27% family then increased to 52% of orders). Four families exhibited paired first leaves only and more than five species: Rubiaceae (Gentianales 6), Loranthaceae (Santalales 7), Caryophyllaceae (Caryophyllales 9), Casuarinaceae (Fagales 9). Three families exhibited single first leaves only and more than five species: Apiaceae (Apiales, 12), Polygonaceae (a.u., 8) and Vitaceae (a.u., 6).

Hypocotyl type

Nearly 80% of species (442 of 567) had glabrous hypocotyls. The proportion of taxa with glabrous hypocotyls only decreased monotonically to 33% at order level, with % polymorphy showing the opposite trend. There was a peak at family level for % taxa with hairy hypocotyls. Caryophyllales (36 spp), Ranunculales (5), Caryophyllaceae (9) and Chenopodiaceae (17; Caryophyllales) were the only orders or families with glabrous hypocotyls only and five or more species sampled. No orders represented by more than one species had hairy hypocotyls only, while 14 of 18 families united in this trait had one species only and none had more than four.

Cotyledon type

The trend in cotyledon type was almost identical to that of hypocotyl type. Eighty four per cent of species had glabrous cotyledons, with the proportion of taxa showing this state decreasing to 41% at order level, and the proportion of polymorphic taxa increasing to 51% of orders. There was a peak at family level for % taxa with hairy cotyledons. Orders and families with glabrous cotyledons only and more than five species were Fagales (11 species), Ranunculales (5), Sapindaceae (Sapindales 8), Casuarinaceae (Fagales 11), Vitaceae (a.u., 6) and Chenopodiaceae (Caryophyllales 17). No orders or families represented by more than five species had hairy cotyledons only.

Embryo type

Sixteen per cent of species (47 of 296) had green embryos (34 genera in 21 families and 13 orders). The proportion of taxa with green embryos-only dropped to 2% of genera, peaked at 20% of families, but dropped again to 7% of orders. The percentage of polymorphic taxa was approximately equal for genera and families (21%), rising to 41% of orders. All orders and families with exclusively green embryos had less than 4 species. Orders and families with exclusively colourless embryos and five or more species: Apiales (6 spp), Asterales (19), Proteaceae (5), Asteraceae (Asterales, 14) and Lauraceae (Laurales, 88).

The peaks in the proportion of character state 2-only families seen in first node type, hypocotyl type, cotyledon type and embryo type are difficult to interpret. It is most likely that they are sampling artifacts due to the high number of families sampled containing very few species.

TABLE 2. Pairwise Pearson correlations (r) between the six binary characters and log seed volume. Correlation significant (2-tailed) $P < 0.001^{***}$, $0.001 < P < 0.01^{**}$, $0.01 < P < 0.05^*$. Number of species in parentheses. Germ: germination type; leaf: first leaf type; node: first node type; hypo: hypocotyl type; coty: cotyledon type; emb: embryo type

	germ	leaf	node	hypo	coty	emb	logvol
germ	1.0 (1738)						
leaf	0.863*** (939)	1.0 (940)					
node	0.046 (840)	0.037 (831)	1.0 (841)				
hypo	-0.020 (567)	-0.024 (495)	0.031 (487)	1.0 (567)			
coty	0.027 (586)	-0.046 (485)	-0.105* (476)	0.450*** (531)	1.0 (586)		
emb	0.005 (291)	-0.046 (242)	-0.091 (158)	0.221** (153)	0.142 (153)	1.0 (296)	
logvol	0.728*** (300)	0.677*** (269)	0.075 (262)	0.106 (198)	0.189** (195)	0.206* (108)	1.0 (301)

Cross-species relationships between the variables

Binary traits

All 60 possible pairwise combinations of binary characters (15 character combinations \times 4 possible states for any pair) occurred in the data, indicating that a particular state for one attribute did not preclude any character state in any other attribute.

Cross-species correlations (Table 2) indicated significant associations between (1) germination and first leaf type: phanerocotily was associated with leaf-like first leaves, while cryptocotily was associated with scale-like first leaves ($r = 0.87$, $P < 0.001$); (2) cotyledon and first node type: glabrous cotyledons were associated with paired first leaves while hairy cotyledons were associated with single first leaves ($r = -0.11$, $P = 0.022$; i.e. the relationship was negative as coded); (3) cotyledon and hypocotyl type: glabrous hypocotyls and glabrous cotyledons were associated, and hairy hypocotyls and cotyledons were associated ($r = 0.45$, $P < 0.001$); (4) embryo and hypocotyl type: colourless embryos were associated with glabrous hypocotyls while green embryos were associated with hairy hypocotyls ($r = 0.22$, $P = 0.006$); (5) embryo and cotyledon type: colourless embryos were marginally associated with glabrous cotyledons while green embryos were associated with hairy cotyledons ($r = 0.14$, $P = 0.08$). A χ^2 test for association between the binary characters designated the same five relationships as significant (with identical P values).

Seed volume versus binary traits

Log seed volume was significantly correlated with (1) germination type: larger seeded species tended to have cryptocotylar germination while smaller seeded species had phanerocotylar germination ($r = 0.73$, $P < 0.001$); (2) first leaf type: larger seeded species tended to have scale-like first leaves ($r = 0.68$, $P < 0.001$); (3) cotyledon type: larger seeds tended to have hairy cotyledons ($r = 0.19$, $P = 0.008$); and (4) embryo type: larger seeds tended to have green embryos ($r = 0.21$, $P = 0.032$). No relationship was found between seed volume and either first node type or hypocotyl type.

TABLE 3. Cross-species ('tip') linear regression versus phylogenetic regression ('phylo'), for qualitative attribute-pairs where the slope (β) of one or the other relationship was significantly different from zero at $\alpha < 0.05$ ($P < 0.001$ ***, $0.001 < P < 0.01$ ** , $0.01 < P < 0.05$ *)

test variable	y variable	tip				phylo			
		β	r^2	P	df	β	r^2	P	df
germination (phanero/crypto)	first leaf (leaf/scale)	0.87	0.75	<0.001***	937	0.65	0.51	<0.001***	93
hypocotyl (glabrous/hairy)	cotyledon (glabrous/hairy)	0.33	0.20	<0.001***	529	0.28	0.55	<0.001***	80
embryo (colourless/green)	hypocotyl (glabrous/hairy)	0.22	0.05	0.006**	151	0.15	0.05	0.10	50
cotyledon (glabrous/hairy)	first node (single/paired)	-0.17	0.01	0.022*	474	-0.004	0.0004	0.10	114
first node (single/paired)	first leaf (leaf/scale)	0.03	0.001	0.281	829	-0.06	0.07	$0.01 < P < 0.025$ *	86
first leaf (leaf/scale)	embryo (colourless/green)	-0.03	0.002	0.479	240	0.17	0.12	$0.025 < P < 0.05$ *	35

Fruit type

Differences between the nine fruit groups with respect to the binary characters were tested with a contingency test (Appendix 2). Highly significant between-group differences ($P \leq 0.001$) were found for all characters except cotyledon type ($P = 0.027$). Differences between fruit types with respect to seed volume also emerged, with approximately 46% of variance in log seed volume explained by differences between groups (ANOVA $P < 0.001$, $\text{Eta}^2 = 0.457$).

Cross-species versus phylogenetic regressions

Binary traits

Two trait/trait-shift relationships were significant in both cross-species and phylogenetic analyses. Divergences in germination mode and first leaf type were strongly associated ($r^2 = 0.51$, $P < 0.001$), such that shifts from phanerocotly to cryptocotly were associated with shifts from leaf-like to scale-like first leaves or, equally, shifts from cryptocotly to phanerocotly were associated with shifts from scale-like to leaf-like first leaves. For brevity, hereafter we shall simply state one direction of attribute-shift but, of course, the other is always implied. Germination mode and first leaf type were also strongly (positively) associated across-species, in fact with greater predictive power ($r^2 = 0.75$, $P < 0.001$). Divergences in cotyledon and hypocotyl type were associated ($r^2 = 0.55$, $P < 0.001$), such that shifts from glabrous to hairy hypocotyls were associated with shifts from glabrous to hairy cotyledons; across-species these traits were also strongly associated, but with less predictive power ($r^2 = 0.20$, $P < 0.001$).

Two trait/trait-shift relationships were significant in cross-species but not in phylogenetic analyses (Table 3), both with low predictive power: embryo type vs. hypocotyl type ($r^2 = 0.05$, $P = 0.006$) and cotyledon type vs. first node type ($r^2 = 0.01$, $P = 0.022$). In addition, the marginally significant cross-species relationship between embryo type and cotyledon type was non-significant in the phylogenetic analysis.

TABLE 4. Cross-species ('tip') linear regression versus phylogenetic regression ('phylo'), for qualitative attributes regressed against log seed volume ($P < 0.001$ ***, $0.001 < P < 0.01$ ***, $0.01 < P < 0.05$ *)

y variable	tip				phylo			
	β	r^2	P	df	β	r^2	P	df
germination (phanero/crypto)	0.21	0.53	<0.001***	299	0.09	0.67	<0.001***	32
first leaf (leaf/scale)	0.20	0.46	<0.001***	268	0.08	0.51	<0.001***	29
embryo (colourless/green)	0.04	0.04	0.032*	107	0.07	0.29	$0.005 < P < 0.01$ **	24
cotyledon (glabrous/hairy)	0.05	0.04	0.008**	194	0.04	0.048	0.10	37
first node (single/paired)	0.02	0.01	0.226	261	-0.005	0.0008	0.10	60
hypocotyl (glabrous/hairy)	0.03	0.01	0.139	197	0.03	0.017	0.10	49

Two trait/trait-shift relationships were significant in phylogenetic but not in cross-species analyses: shifts from paired to single first nodes were associated with shifts from leaf-like to scale-like first leaves ($r^2 = 0.07$, $0.01 < P < 0.025$); shifts from leaf-like to scale-like first leaves were associated with shifts from green to colourless embryos ($r^2 = 0.12$, $0.025 < P < 0.05$). Again, explanatory power was fairly low.

Seed volume versus binary traits

Three trait/trait-shift relationships were significant in both cross-species and phylogenetic analyses (Table 4). Across-species, a significant positive relationship was found between seed volume and germination type ($r^2 = 0.53$, $P < 0.001$; Fig. 2). Similarly, divergences in seed volume and germination mode were strongly associated ($r^2 = 0.67$, $P < 0.001$) such that shifts from phanerocotylar to cryptocotylar germination were linked with increasing seed size. Across-species, a significant positive relationship was found between seed volume and first leaf type ($r^2 = 0.46$, $P < 0.001$). Similarly, divergences in seed volume and first leaf type were strongly associated ($r^2 = 0.51$, $P < 0.001$) such that shifts from leaf-like to scale-like first leaves were linked with increasing seed size. Across-species, seed volume and embryo colour were weakly associated ($r^2 = 0.04$, $P = 0.032$) while divergences in these attributes were more strongly linked ($r^2 = 0.29$, $0.005 < P < 0.010$), such that shifts from colourless to green embryos were linked with positive shifts in seed size.

Across-species, seed volume and cotyledon type were weakly but significantly associated ($r^2 = 0.04$, $P = 0.008$) while divergences in these attributes were not. No relationship was found between seed volume and first node type or hypocotyl type in either set of analyses.

Relationship between seed volume, germination type and first leaf type

Since trends in seed volume, germination type and first leaf type were linked in cross-species and phylogenetic analyses, the relationship between these three variables was further explored (Fig. 3). For species where seed volume data were available, germination type and first leaf type were strongly correlated ($r = 0.87$, $P < 0.001$, $n = 270$), just as in the full data set. This relationship was weakened only slightly when

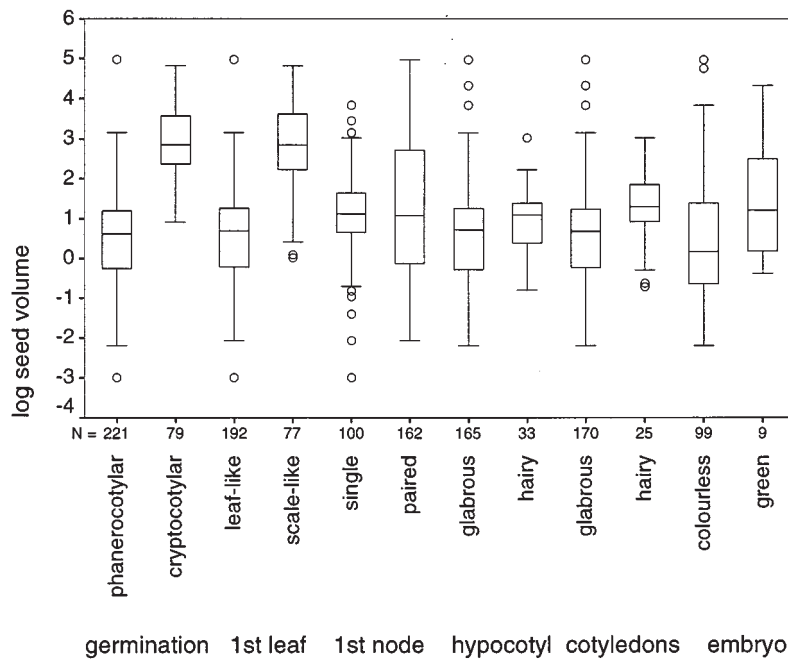


Figure 2. Range in log seed volume for binary character states. Boxplots show median and interquartile range; circles indicate outliers (cases with values >1.5 box lengths from upper or lower edge of box).

re-tested with log seed volume controlled for (partial correlation $r=0.75$, $P<0.001$). Similar results were obtained in phylogenetic regressions of germination type against first leaf type (and vice versa) for the seed volume data subset. That is, a strong relationship between divergences in germination type and first leaf type was evident whether or not divergences in seed volume were controlled for. In summary, it was clear that the relationship between germination mode and first leaf type cannot be attributed to a secondary correlation with seed size.

By contrast, across species and within lineages the association between seed size and first leaf type could be a secondary correlation due to those between seed size and germination type, and germination and first leaf type. The partial correlation of log seed volume and germination type, controlling for first leaf type, was still significant ($r=0.413$, $P<0.001$) while that between log seed volume and first leaf type (controlling for germination type) was not ($r=0.098$, $P=0.110$). Phylogenetic regression of germination type against log seed volume while controlling for first leaf type was significant ($r^2=0.45$, $P>0.001$), while that between first leaf type against log seed volume while controlling for germination type was significant yet much weaker ($r^2=0.13$, $0.025<P<0.05$).

DISCUSSION

Taxonomic survey of attributes

Germination type

Approximately 22% of 1738 species surveyed exhibited cryptocotylar germination. These species were spread right across the phylogenetic tree, representing 124 genera,

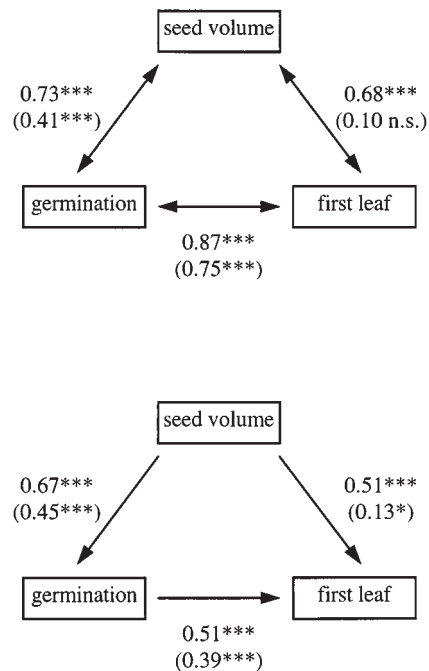


Figure 3. Path diagram for germination type – first leaf type – log seed volume trait interrelationships for data subset for which seed volume was recorded (302 species). Top: cross-species bivariate correlation r values, and partial correlation r values (in parentheses, controlling for third variable). Below: r^2 values from phylogenetic regressions, with arrows pointing from test (predictor) to y variables. Again, values given in parentheses refer to strength of association with third variable controlled for. *** $P < 0.001$; * $0.025 < P < 0.05$; n.s. $P < 0.05$.

40 families and 24 orders. Germination type did not appear to be ‘taxonomically conservative’, with 16%, 25% and 42% of genera, families and orders respectively recorded here as polymorphic. Few higher taxa contained only cryptocotylar species. The two orders listed here as containing only cryptocotylar species in fact only had one species apiece; furthermore, one of them (Nymphaeales) has elsewhere been recorded to contain phanerocotylar species (Clifford, 1991). Lauraceae (91 species) on the other hand may indeed only contain cryptocotylar species, on comparison of our data with those published elsewhere. Since phanerocotily is the more common state across all species (here 78% of 1738 spp; 86% of 3661 spp: Clifford, 1991; 64% of 200 Malaysian forest tree species: Ng, 1978; 83% of 81 Taiwanese rainforest species: Li & Hsieh, 1997) it was not surprising that a number of higher taxa were exclusively phanerocotylar. However, comparison of our data with those of Clifford (1991) and Burt (1991) indicate that Caryophyllales may be one of the few taxa that fulfill this description (e.g. Capparales, Cornales, Elaeocarpaceae, Fagales, Icacinaceae, Polygonaceae, Primulales, Ranunculales and Rhamnales—listed here as phanerocotylar only—all contain at least one cryptocotylar species). Similarly, Clifford (1991) suggested that Asteriflorae had no known cryptocotylar species yet one such species was recorded here in Asteraceae (*Jaegera dasyantha*).

Previous studies have attempted to determine which germination mode was

ancestral and which was derived. For example, Burt's (1991) stated interest was in "showing that cryptocotyly has arisen repeatedly from phanerocotyly in very many different families." Burt (1991) cited a number of studies besides his own where 'advanced' taxa were cryptocotylar while 'primitive' taxa were phanerocotylar. By contrast, Clifford (1984, 1991) demonstrated that the proportion of cryptocotylar species is generally greater in 'primitive' taxa such as the Magnolids than in 'advanced' taxa such as Asteriflorae (*sensu* Dahlgren, 1980), and suggested that this provided evidence that phanerocotyly is the derived condition and cryptocotyly the ancestral state (a view also shared by Grushvitskiy, 1963 and de Vogel, 1980). Burt (1991), aware of this fact also, suggested that this evidence was misleading since families allocated 'advanced' status are predominantly small-seeded and herbaceous species, and among these cryptocotyly is rare.

Our data indicated that there have been many independent evolutionary divergences between cryptocotyly and phanerocotyly in this sample of Australian dicots, and that a shift from one germination mode to the other is not necessarily irreversible within a lineage. For example, in Fabales there appears to have been at least nine independent shifts from phanerocotyly to cryptocotyly and three independent reversals. Similarly, in Proteaceae four independent shifts from phanerocotyly to cryptocotyly and three independent reversals were apparent (reconstruction of ancestral character states by parsimony). This suggests that the debate over which condition is primitive and which is advanced will not have any general answer, only an answer for a specific branch-step in the phylogenetic tree.

Embryo colour

Approximately 16% of 296 species had green embryos. These species had a wide taxonomic spread, coming from 34 genera in 21 families and 13 orders. Previous surveys are few in number. Yakovlev & Zhukova (1980) reported the occurrence of chlorophyllous embryos in 428 angiosperm species (224 genera, 72 families) in a sample of 1094 species (666 genera, 182 families). The lower proportion of species with green embryos in our sample may be due in part to different methodology: here embryo colour was noted in mature seeds only, while Yakovlev & Zhukova (1980) defined chloroembryophytes as species with seeds which have chlorophyll present at any stage during embryogenesis. Janzen (1982) surveyed seeds of 74 Costa Rican species (34 families), finding 55% of species were chlorophyllous (from 15 families). Although this character has previously been suggested to be taxonomically conservative (Dahlgren, 1980; Yakovlev & Zhukova, 1980), our data do not support this view with no well-represented families or orders recorded as exclusively chlorophyllous, and 22% of genera, 21% of families and 41% of orders polymorphic in embryo colour. Janzen (1982) also found 21% of families to be polymorphic for this character.

Here, of the few higher taxa with green embryo-species only, Santalales and Verbenaceae have elsewhere been shown to include species in conflict with this result (Yakovlev & Zhukova, 1980; Janzen, 1982). Similarly, Yakovlev & Zhukova (1980) reported that all species in, among others, Brassicaceae, Rhamnaceae and Fabales had green embryos, yet we found colourless embryo species in these three groups (although our data may not conflict with theirs if chlorophyll had been present in the immature seed). Capparales, Gentianales and Malvales—found here to include species with colourless embryos only—are in fact polymorphic (Yakovlev

& Zhukova, 1980; Janzen, 1982), while Fagales (reported to have colourless embryos by Yakovlev & Zhukova 1980) was found here to be polymorphic. Apiales, Asterales and Lauraceae may in fact be uniform in only containing species with colourless embryos.

Other attributes

We are not aware of systematic surveys of first leaf type or phyllotaxy, nor of the presence of hairs on cotyledons and hypocotyls. Like germination type and embryo colour, our survey suggests that polymorphism in these characters at genus level and above is common and that shifts from one character state to another have occurred frequently throughout the evolutionary history of the dicots.

Correlated traits and trait shifts

Although the sample size for seed volume was small relative to that of most other primary attributes, discussing trait relationships with respect to seed size is profitable since it is a pivotal trait in a species' seedling establishment strategy (Westoby, Leishman & Lord, 1996). Seed size has been shown experimentally to be negatively correlated with seedling potential relative growth rate RGR, specific leaf area SLA (of cotyledons, and of leaves) and specific root length SRL (e.g. Jurado & Westoby, 1992; Marañón & Grubb, 1993; Kitajima, 1992; Wright & Westoby, 1999). That is, small seeded species tend to exhibit a syndrome of leaf and root surface maximisation which enables them to achieve high RGR, provided that they are establishing in a site where light, water and nutrients are not severely limiting. Since species with smaller seeds can produce more seeds per unit reproductive effort than larger seeded species, they can be expected to disperse propagules to a greater number of potential establishment sites (although the link between distance dispersal and seed size is not simple: Hughes *et al.*, 1994). On the other hand, in the face of hazards such as deep shade, defoliation, and nutrient or water shortage, large seeded species tend to out-perform smaller seeded species since they are better provisioned with energy reserves (reviewed in Westoby *et al.*, 1996).

Germination type and seed size

Across-species, phanerocotylly was associated with leaf-like first leaves and cryptocotylly associated with scale-like first leaves; similarly, within-lineages there was a strong tendency for shifts in the two traits to be correlated. Within-lineages, shifts in germination mode and first leaf type were strongly associated with shifts in seed volume and, across-species, log seed volume predicted germination mode and first leaf type better than any other traits. While the relationship between germination mode and first leaf type was independent of that between either character and seed size, the relationship between seed volume and first leaf type was apparently due to those between seed size and germination type, and germination type and first leaf type, rather than directly leading from seed size.

Although all seeds with volume less than *c.* 8 mm³ were phanerocotylar (Fig.2), not all large seeded species had cryptocotylar germination (in fact the largest seeded species, *Aleurites molucanna* (Euphorbiaceae) was phanerocotylar). Ng (1978) found a

more clear-cut distinction in Malaysian forest trees, where all species with phanerocotylar (and epigeal) germination had seeds less than 3 mm in length, and all seeds 60 mm or longer were semihypogeal or hypogeal.

Large-seeded species with abundant stored reserves face a number of strategic 'decisions' as to where the reserves should be situated (e.g. endosperm vs cotyledons) and how they are protected. Firstly, for seedlings in which the cotyledons are primarily storage organs rather than photosynthetic organs (e.g. many large seeded species) there may be little point in withdrawing the cotyledons from the testa; in fact the protective benefit of cryptocotily may outweigh any photosynthetic gain from having the cotyledons exposed. Secondly, large-seeded species must also make a strategic 'decision' as to whether the cotyledons should remain at or below ground level following germination, or whether they should be raised above ground by extension of the hypocotyl. In principle, two factors may be important here: energetic/structural considerations and herbivore pressure.

Lifting bulky cotyledons above the ground would involve energetic costs which could be avoided by adopting the hypogeal condition. Direct costs include producing and maintaining a suitably strong extended hypocotyl; further costs may include producing a root system capable of supporting a structure with high centre of gravity. It is not known to what extent the type and severity of herbivore pressure differs for epigeal and hypogeal seedlings, however some lines of evidence suggest that the hypogeal condition affords protection against large herbivores. In horse chestnut (*Aesculus turbinata*), for example, stored resources in hypogeal, cryptocotylar cotyledons appear to work as a 'bet hedge' against severe above-ground clipping by rodents (Kazuhiko, Suzuki & Satohiko, 1997). In a similar vein, by burying potted seedlings in Australian rainforests it has been shown that birds scratch through and turn over the litter about once a fortnight and in the process damage the seedlings (H.T. Clifford, unpublished data). Whether or not the seedlings are also grazed has not been determined.

Thus increases in seed size may have been the starting point for shifts from epigeal phanerocotily to hypogeal cryptocotily (and perhaps a decrease in seed size could initiate selective pressure for the opposite sequence of events). Such shifts may have occurred in two stages, with either sequence (shift to hypogeally followed by shift to cryptocotily, or vice versa) possible in principle. Indeed, a small number of species exhibit the possible 'intermediate' conditions in this scheme (e.g. *Lobelia*: hypogeal and phanerocotylar; *Alyxia ruscifolia*: cryptocotylar and epigeal). An increase in seed size due to an enhanced cotyledonary storage role (with concomitant decrease in the photosynthetic role of the cotyledons) could be followed by a shift to cryptocotily (protection) and a secondary shift to hypogeally (further protection; structural considerations). Alternatively, an increase in seed size (again, due to increased cotyledon size) could be followed by a shift to hypogeal cotyledons (protection; structural considerations) with little or no opportunity for photosynthesis. In such a situation, the loss of cotyledonary photosynthetic potential and development of cryptocotily would seem advantageous. This second scenario seems more likely to occur in shaded or understorey situations where the cotyledons would receive little light if not lifted by the hypocotyl. While habitat was not used as a variable in this study, it is worth noting that cryptocotily is probably most common in seedlings of rainforest tree species where it is associated with large seeds and germination in low illumination (Clifford, 1984; Burt, 1991). Still, cryptocotily is ecologically widespread, being known from a variety of growth forms in heathlands, mangrove

forests and rainforests, and from both small and large seeded species (Clifford, 1984). Thus, it is possible that many developmental sequences have led to its broad occurrence.

An alternative sequence of events leading to the correlation of cryptocotily and seed size involves increased endosperm rather than cotyledon mass. According to Burt (1991), while in phanerocotylar seedlings the absorption of endosperm by haustorial cotyledons is completed by the time the seed is ready to germinate (thus the cotyledons may then take on a photosynthetic role), with an increase in endosperm it sometimes happens that the correlation of timing breaks down and the seed is ready to germinate before all the endosperm is absorbed. When this happens, either the cotyledons remain inside the seed-coat as haustorial organs, or part of the endosperm is wasted. The replacement of endosperm by food-storing cryptocotylar cotyledons may be complete, as in Lauraceae, or the endosperm may be absorbed by permanently haustorial cotyledons, as in Annonaceae or Myristicaceae (Burt 1991). However, this scenario seems unlikely since in cryptocotylar species the site of reserve storage is generally the cotyledons rather than in an endosperm: of 276 cryptocotylar species for which we could confidently predict the presence or absence of endosperm from family affinity (data from Clifford & Ludlow, 1978), 241 lacked endosperm. Clifford (1991) reported a similar association.

First leaf type

While the frequent occurrence of cataphyllous first leaves in hypogeal, cryptocotylar species has been reported previously (Duke, 1969; Ng, 1978; de Vogel, 1980; Godley, 1985; Clifford, 1991), the function of these scale-like leaves is unclear. Removing the apical meristem from several rainforest species with cataphylls resulted in buds expanding from their axils in all cases (H.T. Clifford, unpublished data). Thus cataphylls may provide a source of buds without placing a high respiration load on the seedling which would result if a lamina developed. In poor light such a lamina would achieve little photosynthesis but would consume a fair amount of respiration energy. By contrast, de Vogel (1980) suggested that cataphylls may have a protective function. In hypogeal species the epicotyl rather than the hypocotyl elongates; perhaps formation of cataphylls protects the delicate apical meristem from physical damage during upwards movement through the soil (in addition to the protection already afforded by the plumules).

Embryo colour

The significance of embryo chlorophyll is somewhat unclear. It is relatively common for chlorophyll and Calvin Cycle enzymes to occur in embryo (and cotyledon) chloroplasts, but in many species chlorophyll content declines and chloroplast structure becomes disrupted by the time of seed maturation (Rascio, Vecchia & Casadoro, 1990; Eastmond, Kolácná & Rawsthorne, 1996). For example, all of the chloroembryophytes reported by Janzen (1982) had achlorophyllous embryos in the mature or dormant seeds. In *Brassica napus*, approximately 30% of incident light is transmitted through the silique wall, suggesting that *c.* 1500 $\mu\text{mol.m}^{-2}.\text{sec}^{-1}$ external PAR would be required for light compensation point in the chlorophyllous embryos (Eastmond *et al.*, 1996). In this species, although very little carbon is fixed by embryo chloroplasts, significant electron transport has been detected (Asokanathan *et al.*, 1997). It seems likely then that the photoheterotrophic

embryos may use the light reactions to generate the ATP and NADPH required to fuel the conversion of maternally supplied sucrose to the fatty acids used in oil synthesis and storage (Asokanthan *et al.*, 1997). In canola this seems highly plausible since the seeds are rich in oils; indeed, they have been selectively bred for this trait.

Published data on seed oil content was available for very few of our study species, but data were available for 41 families for which we had records of embryo colour (Appendix 1). No relationship was found between oil content and embryo colour in the comparison between our data and family means, nor at species level when each species was assigned its average family oil content (correlation $r=0.10$, $P=0.114$). Families with colourless embryos exclusively had between 0.04% (Aizoaceae) and 66.7% (Goodeniaceae) oil content (mean 24.7, SD 17.1, $n=26$); polymorphic families had between 5% (Myrtaceae) and 57.7% (Meliaceae) oil (mean 28.3, SD 19.5, $n=10$); and families exclusively with chlorophyllous embryos had between 9% (Cunoniaceae) and 25.2% (Verbenaceae) oil content (mean 16.9, SD 7.1, $n=5$). ANOVA for difference between groups in oil content was non-significant ($P=0.478$).

For light to reach embryo chlorophyll it must penetrate the maternal fruit tissues and other surrounding seed tissues such as cotyledons and endosperm, if present. Dahlgren (1980) reported a tendency for chlorophyllous embryos to occur in non-endospermous seeds, suggesting that the presence of endosperm substantially interferes with the transmittance of light to the developing embryo. By contrast, of 28 chlorophyllous species for which we had endosperm data, 13 had endosperm and 15 did not. In Costa Rican deciduous forest species, chlorophyllous embryos were found in species with strongly insolated developing fruits, fruits with all the seeds about equally close to the surface, and fruits and seeds thin enough that light could presumably penetrate to the embryo (Janzen, 1982). Achlorophyllous seeds were found in fruits with large seeds, fruits with many seeds packed in layers, or in fruits borne in locally shady habitats. While embryo and fruit type were strongly associated ($P<0.001$; Appendix 2), colourless embryos were found in seeds from all fruit types and green embryos in seeds from all fruit types but cypselas and nuts. It is unlikely that light could penetrate through the fruit wall in nuts, but then again the same could generally be said of capsules, and 29 of 114 capsular fruit contained seeds with green embryos. In contrast, intuitively one would expect light to penetrate the cypselas of many Asterids.

Here, seed size and embryo colour were only weakly associated across species, yet log seed volume was a strong predictor of embryo colour within-lineages ($r^2=0.29$, $0.005<P<0.010$) such that upward shifts in seed size were associated in shifts from colourless to green embryos. The production of larger seeds must result in fewer seeds being produced per unit reproductive effort, and the advantage in producing seeds with chlorophyllous embryos is presumably in being able to provision seeds more than would be possible otherwise. But, depending on how much more costly seeds which develop chlorophyllous embryos are to produce than those without chlorophyll (if, indeed, they are more costly), it is also a case of the seeds provisioning themselves. This adds further variables to the already complex host-parasite interaction between fruit and seeds. For example, the benefits conferred by a fruit shape and fruit coat translucence which enable seed embryos to receive light would have to be balanced against the effects of changing fruit morphology on seed dispersal by frugivores. All benefits would also have to be balanced against the loss of control of the embryo by the parent (Janzen, 1982).

Embryo type was also variously associated with hypocotyl type, cotyledon type

and first leaf type. The possibility that these relationships were secondary correlates of relationships with seed volume was explored by controlling for seed volume in cross-species partial correlations and phylogenetic regressions. In all cases, the original bivariate relationships were weakened only marginally (results not shown). Since chlorophyll synthesis in the developing embryo is not known to not confer any clear advantage during germination or seedling growth (Rascio *et al.*, 1990), we offer no prospective explanations for these relationships.

Cotyledon type, hypocotyl type and seed size

Species with glabrous hypocotyls tended to have glabrous cotyledons and those with hairy hypocotyls tended to have hairy cotyledons, although this was not always true (correlation $r=0.45$, $P<0.001$). Within lineages, shifts in the two characters were more strongly associated. Across-species there was a tendency for larger seeded species to have hairy cotyledons, while divergences in the two attributes were not associated within lineages. The relationships between hypocotyl type and seed size, and shifts in the two characters within-lineages, were not significant.

Hairs on plant surfaces may function in defence (particularly if they are glandular hairs, such as in *Drosera* or in Solanaceae), or in creating a thicker boundary layer and thus decreasing evaporation (from leaf surfaces, in particular, but presumably also from any parts of a desiccation-prone seedling). Depending on the function of cotyledonary hairs, one can predict alternative relationships with seed size. Small seeds are poorly equipped for survival in the face of hazards such as shade and, not surprisingly, species from open-habitats (whether always open, or having an open regeneration-niche) have smaller seeds, on average (Salisbury, 1942, 1974; Foster & Janson, 1985; Mazer, 1989; Thompson & Hodgkinson, 1998; but see Kelly, 1996). Since the risk of desiccation is greater in open than in shaded habitats, the presence of hairs which reduce evapotranspiration could be expected to be more common in species found in such places, and thus of smaller seeded species in general. In contrast, one would expect that hairs which operate primarily in seedling defence would be more common in large seeded species, since (1) they have a greater storage reserve to protect than smaller seeds and (2) it is perhaps less crucial to allocate the maximum resources possible to the photoassimilation of carbon. The result—that cotyledonary hairs are more common in large seeded species—supports the defence hypothesis and not the desiccation hypothesis. However, since hairs were recorded only as present or absent, we cannot draw any conclusions on the matter.

Conclusions

All possible pairwise combinations of the six binary characters were found in our sample of Australian dicot species, indicating that a particular state for one attribute did not preclude any character state in any other attribute. The less common attribute states for germination mode, first leaf type, hypocotyl texture, cotyledon texture and embryo colour occurred in species from right across the dicotyledon phylogeny, as did independent evolutionary divergences in each of these characters. Although the occurrence of particular attribute states was characteristic of various taxa, in general attribute polymorphy was common at genus level and above. With

the exception of the associations between germination mode and first leaf type, and cotyledon and hypocotyl type, cross-species regressions between the binary attributes had low predictive power, even where statistically significant. Taken together, these lines of evidence suggest that the traits have assorted more or less independently of each other and provide no evidence of functional groups based on these attributes.

Still, a number of trait pairs were strongly associated in both cross-species (correlated-occurrence) and phylogenetic (correlated-divergence) analyses. In particular, cryptocotly, scale-like first leaves and large seed volume were associated, as were hypocotyl and cotyledon type. Large seed volume and the presence of green embryos were strongly associated in phylogenetic analyses but only weakly in cross-species analyses. The large sample size and high degree of statistical association of these attribute relationships indicated that they are relationships which have been commonly linked throughout Australian dicot evolution.

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REFERENCES

- Asokanathan PS, Johnson RW, Griffith M, Krol M. 1997.** The photosynthetic potential of canola embryos. *Physiologia Plantarum* **101**: 353–360.
- Barclay AS, Earle FR. 1974.** Chemical analyses of seeds III. Oil and protein content of 1253 species. *Economic Botany* **28**: 178–236.
- Burtt BL. 1991.** On cryptocotylar germination in dicotyledons. *Botanische Jahrbucher fur Systematik, Pflanzengeschichte und Pflanzengeographie* **113**: 429–442.
- Clifford HT. 1984.** Cryptocotly in Australian dicotyledons. *Flora Malesiana Bulletin* **37**: 49–53.
- Clifford HT. 1987.** Identification of seedlings in the Australian flora. In: Langkamp P, ed. *Germination of Australian Plant Seed*, Melbourne: Inkata Press, 98–106, 224–231.
- Clifford HT. 1991.** Germination patterns in dicotyledons. *ALISO* **13**: 207–213.
- Clifford HT, Ludlow G. 1978.** *Keys to the families and genera of Queensland flowering plants (Magnoliophyta)*, 2nd edition. St. Lucia: University of Queensland Press.
- Dahlgren R. 1980.** The taxonomic significance of chlorophyllous embryos in angiosperm seeds. *Botaniska Notiser* **133**: 337–341.
- de Vogel EF. 1980.** *Seedlings of dicotyledons*. Wageningen, The Netherlands: Centre for Agricultural Publishing and Documentation.
- Duke JA. 1965.** Keys for the identification of seedlings of some prominent woody species in 8 forest types in Puerto Rico. *Annals of the Missouri Botanical Garden* **52**: 314–350.
- Duke JA. 1969.** On tropical tree seedlings. 1. Seeds, seedlings, systems, and systematics. *Annals of the Missouri Botanical Garden* **56**: 125–161.
- Earle FR, Jones Q. 1962.** Analyses of seed samples from 113 plant families. *Economic Botany* **16**: 221–250.
- Eastmond P, Koláčná L, Rawsthorne S. 1996.** Photosynthesis by developing embryos of oilseed rape (*Brassica napus* L.). *Journal of Experimental Botany* **47**: 1763–1769.
- Flora of Australia. 1981.** Canberra: Bureau of Flora and Fauna.

- Foster SA, Janson CH. 1985.** The relationship between seed size and establishment conditions in tropical woody plants. *Ecology* **66**: 773–780.
- Garwood NC. 1995.** Functional morphology of tropical tree seedlings. In: Swaine MD, ed. *The Ecology of Tropical Forest Tree Seedlings*. New York: Parthenon, 59–129.
- Godley EJ. 1985.** Paths to maturity. *New Zealand Journal of Botany* **23**: 687–706.
- Grafen A. 1989.** The phylogenetic regression. *Philosophical Transactions of the Royal Society of London B* **326**: 119–157.
- Grafen A. 1992.** The uniqueness of the phylogenetic regression. *Journal of Theoretical Biology* **156**: 405–423.
- Grafen A, Ridley M. 1996.** Statistical tests for discrete cross-species data. *Journal of Theoretical Biology* **183**: 255–267.
- Grushvitskiy IV. 1963.** Subterranean germination and the function of the cotyledons. *Botanicheskii zhurnal* **48**: 906–915.
- Harden GJ. 1990.** *Flora of NSW*. Sydney: Royal Botanical Gardens.
- Hladik A, Miquel S. 1990.** Seedling types and plant establishment in an African rain forest. In: Bawa KS, Hadley M, eds. *Reproductive Ecology of Tropical Plants*. Paris/Carnforth: UNESCO/Parthenon, 261–282.
- Hughes L, Dunlop M, French K, Leishman MR, Rice B, Rodgerson L, Westoby M. 1994.** Predicting dispersal spectra: a minimal set of hypotheses based on plant attributes. *Journal of Ecology* **82**: 933–950.
- Hyland BPM, Whiffin T. 1993.** *Australian tropical rain forest trees: an interactive identification system*. East Melbourne, Victoria: CSIRO Australia.
- Janzen DH. 1982.** Ecological distribution of chlorophyllous developing embryos among perennial plants in a tropical deciduous forest. *Biotropica* **14**: 232–236.
- Jones Q, Earle FR. 1966.** Chemical analyses of seed II: Oil and protein content of 759 species. *Economic Botany* **20**: 127–155.
- Jurado E, Westoby M. 1992.** Seedling growth in relation to seed size among species of arid Australia. *Journal of Ecology* **80**: 407–416.
- Kazuhiko H, Suzuki W, Satohiko S. 1997.** Impacts of secondary seed dispersal and herbivory on seedling survival in *Aesculus turbinata*. *Journal of Vegetation Science* **8**: 735–742.
- Kelly CK. 1996.** Seed mass, habitat conditions and taxonomic relatedness: a re-analysis of Salisbury (1974). *New Phytologist* **135**: 169–174.
- Kelly CK, Purvis A. 1993.** Seed size and establishment conditions in tropical trees. *Oecologia* **94**: 356–360.
- Kitajima K. 1992.** Relationship between photosynthesis and thickness of cotyledons for tropical tree species. *Functional Ecology* **6**: 582–589.
- Kitajima K. 1995.** Cotyledon functional morphology, patterns of seed reserve utilization and regeneration niches of tropical tree seedlings. In: Swaine MD, ed. *The Ecology of Tropical Forest Tree Seedlings*. New York: Parthenon, 193–210.
- Levin DA. 1974.** The oil content of seeds: an ecological perspective. *American Naturalist* **108**: 193–206.
- Li S-P, Hsieh C-F. 1997.** Seedling morphology of some woody species in a subtropical rain forest of southern Taiwan. *Taiwania* **42**: 207–238.
- Lord J, Westoby M, Leishman M. 1995.** Seed size and phylogeny in six temperate floras: constraints, niche conservatism, and adaptation. *American Naturalist* **146**: 349–364.
- Marañón T, Grubb PJ. 1993.** Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Functional Ecology* **7**: 591–599.
- Mazer SJ. 1989.** Ecological, taxonomic, and life history correlates of seed mass among Indiana Dune angiosperms. *Ecological Monographs* **59**: 153–175.
- Ng FSP. 1978.** Strategies of establishment in Malayan forest trees. In: Tomlinson TB, Zimmerman HM, ed. *Tropical Trees as Living Systems*. Cambridge: Cambridge University Press, 129–162.
- Price T. 1997.** Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society of London B* **352**: 519–529.
- Rascio N, Vecchia FD, Casadoro G. 1990.** Behaviour of cotyledonal plastids during seed germination in some chloroembryophytes. *Cytobios* **64**: 53–64.
- Ridley M. 1983.** *The explanation of organic diversity*. Oxford: Clarendon Press.
- Salisbury EJ. 1942.** *The reproductive capacity of plants*. London: G. Bell & Sons.
- Salisbury EJ. 1974.** Seed size and mass in relation to environment. *Philosophical Transactions of the Royal Society of London B* **186**: 83–88.

- Thompson K, Hodkinson DJ. 1998.** Seed mass, habitat and life history: a re-analysis of Salisbury (1942, 1974). *New Phytologist* **138**: 163–167.
- Westoby M, Cunningham SA, Fonseca C, Overton J, Wright IJ. 1998.** Phylogeny and variation in light capture area deployed per unit investment in leaves: designs for selecting study species with a view to generalizing. In: Lambers H, Poorter H, van Vuuren MMI, eds. *Variation in Growth Rate and Productivity of Higher Plants*. Leiden, The Netherlands: Backhuys, 539–566.
- Westoby M, Leishman M, Lord J. 1996.** Comparative ecology of seed size and dispersal. *Philosophical Transactions of the Royal Society of London B* **351**: 1309–1318.
- Wright IJ, Westoby M. 1999.** Differences in seedling growth behaviour among species: trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *Journal of Ecology*. **87**(1): 85–97.
- Yakovlev MS, Zhukova GYA. 1980.** Chlorophyll in embryos of angiosperm seeds, a review. *Botaniska Notiser* **133**: 323–336.

APPENDIX 1

List of character states, by family and order. Where taxonomic affinity was uncertain at order level in Uppsala phytogeny, the name of the next highest monophyletic clade is given in parentheses (see Figure 1). Abbreviations germ: germination type (phanerocotylar 1, cryptocotylar 2); leaf: first leaf (leaf-like 1, scale-like 2); node: first node (leaf single 1, leaves paired 2); hypo: hypocotyl (glabrous 1, hairy 2); coty: cotyledons (glabrous 1, hairy 2); emb: embryo (colourless 1, green 2); n1: number species in order; n2: number genera in family; n3: number species in family; 1&2 denotes taxon polymorphic for that character; oil: % oil content reported in literature (see text). Note that *n* values indicate number of species or genera found in data set, but that many species were not measured for all characters.

Order	germ	leaf	node	hypo	coty	emb	n1	Family	germ	leaf	node	hypo	coty	emb	n2	n3	oil
Apiales	1&2	1	1&2	1&2	1&2	1	34	Apiaceae	1&2	1	1	1&2	1&2	1	10	20	19.7
								Pittosporaceae	1	1	1&2	1&2	1&2	1	6	14	13.5
Asterales	1&2	1&2	1&2	1&2	1&2	1	49	Asteraceae	1&2	1	1&2	1&2	1&2	1	30	37	24.9
								Campanulaceae	1	1&2	1&2	1	1	1	3	5	40.7
								Goodeniaceae	1	1	1	1&2	1&2	1	4	6	66.7
								Styidiaceae	1	1	1	1	1	1	1	1	
								Akaniaceae	1	1	1	1	1	1	1	1	
								Bataceae	1	1	1	1	1	1	1	1	
								Brassicaceae	1	1	1&2	1&2	1&2	1	5	6	32.5
								Capparaceae	1	1	2	2	1	1	2	2	
								Limnathaceae	1	1	1	1	1	1	1	1	
								Alzooaceae	1	1	1&2	1	1	1	3	3	0.04
								Amaranthaceae	1	1	1&2	1	1	1&2	3	4	8.1
								Caryophyllaceae	1	1	2	1	1&2	1	8	9	10.3
								Chenopodiaceae	1	1&2	1&2	1	1	1&2	12	20	8.9
								Molluginaceae	1	1	2	1	1	1	1	1	
								Nyctaginaceae	1	1	2	1	1	1	2	2	
								Phytolaccaceae	1	1	1	1	1	1	1	1	
								Portulacaceae	1	1	1	1	1	1	2	2	19.0
								Celastraceae	1&2	1&2	1	1	1	1&2	12	18	51.0
Celastrales	1&2	1&2	1	1	1	1&2	18	Cornaceae	1	1	1	1&2	1	4	4	1	
Cornales	1						1	Cucurbitaceae	1&2	1&2	1	1&2	1	1	1	1	
Cucurbitales	1&2	1&2	1	1&2	1		4	Actinidiaceae	1	2	1	1	1	1	1	6	
Ericales	1&2	1&2	1&2	1	1	1	38	Ebenaceae	1	1	2	1	1	1	4	4	
								Ericaceae	1	1	2	1	1	1	4	4	
								Lecythidaceae	1&2	2					2	5	
								Sapotaceae	1&2	1&2	1&2				6	19	2
								Symplocaceae	1	1					1	1	
								Theaceae	1	1					1	1	

continued

APPENDIX 1—*continued*

Order	germ	leaf	node	hypo	coty	emb	n1	Family	germ	leaf	node	hypo	coty	emb	n2	n3	oil	
Fabales	1&2	1&2	1&2	1&2	1&2	1&2	223	Fabaceae	1&2	1&2	1&2	1&2	1&2	1&2	64	218	7.8	
								Polygalaceae	1	1	1	1	1	1	1	1	1	
								Suraniaceae	1&2	1&2	1	1&2	1&2	2	2	2	2	
Fagales							14	Xanthophyllaceae	1					2	1	2		
								Balanopaceae	1	2	2	1&2	1	2	1	1	1	
								Casuarinaceae	1	1	1	1	1	1	2	11	42.0	
Gentianales	1&2	1&2	1&2	1&2	1&2	1	58	Fagaceae	1	1	1	1	1	1	1	2		
								Apocynaceae	1&2	1&2	1&2	1&2	1&2	16	16	25		
								Gentianaceae	1	1	2	1	1	1	1	1	1	
Geraniales							2	Loganiaceae	1	1&2	2	1	1	1	3	6		
								Rubiaceae	1	1	2	1	1&2	1	18	26	8.6	
								Geraniaceae	1	1	1	2	2	2	2	1	2	14.5
Lamiales	1&2	1	1&2	1&2	1&2	1&2	42	Acanthaceae	1	1	2	1	1	1	2	3	5.0	
								Bignoniaceae	1	1	2	1	1&2	1	2	2	2	
								Byblidaceae	1	1	1	2	2	1	1	1	1	
Laurales								Callitricaceae	1	1	2	1	1	1	2	2	2	
								Gesneriaceae	1	1	2	1	2	2	2	2	2	
								Lamiaceae	1	1	2	2	2	2	3	3	3	
								Myoporaceae	1	1	1&2	1&2	1&2	1&2	1&2	2	4	
								Oleaceae	1&2						5	7		
								Plantaginaceae	1	1	1	1	1	1	1	1	1	6.9
								Scrophulariaceae	1	1	2				1	1	1	
								Verbenaceae	1&2	1	1	2	1&2	2	9	16	25.2	
								Hernandiaceae	1&2	1	1	2	1&2	2	2	5		
								Idiospermaceae	2	2	1			2	1	1	1	
								Lauraceae	2	1&2	1&2	1	1	1	1	8	91	47.3
	Magnoliales							18	Monimiaceae	1	1	2			1	10	16	
								Annonaceae	1&2	1&2	1&2	2	1&2	1	10	14	21.7	
								Eupomatiaceae	1	1	1	1	1	1	1	1	1	
								Himantandraceae	1	1	1	1	1	2	1	1	1	
								Myrsicaceae	1&2	2	1			2	2	2	2	

continued

APPENDIX 1—continued

Order	germ	leaf	node	hypo	coty	emb	n1	Family	germ	leaf	node	hypo	coty	emb	n2	n3	oil	
Malvales	1&2	1&2	1&2	1&2	1&2	1	69	Bixaceae	1	1	1	1	1	1	1	1	5.0	
								Bombacaceae	1	1	1	1	1	1	2	2		
								Malvaceae	1	1	1	1&2	1&2	1&2	1	5	9	
								Sterculiaceae	1&2	1&2	1&2	1&2	1&2	1	11	48	23.8	
								Thymelaeaceae	1&2	1	2	1	1&2	1	5	5		
Myrtales								Tiliaceae	1&2	1	2	2	2	3	4	4		
								Combretaceae	1&2	1	1&2	1	1&2	1	3	10		
	1&2	1&2	1&2	1&2	1&2	1&2	373	Lythraceae	1	1	2	2	1	1	2	2		
								Melastomataceae	1	1	2	1	1	1	1	2	3	
								Myrtaceae	1&2	1&2	1&2	1&2	1&2	1&2	1&2	40	356	5.0
								Onagraceae	1	1	2	1	1	1	1	1	2	26.6
								Nymphaeaceae	2	1	1	1	1	1	1	1	1	
								Cephalotaceae	1	1	1	1	1	1	1	1	1	
								Cunoniaceae	1	1	2	1&2	1&2	2	10	15	9.0	
								Eucryphiaceae	1	1	2	2	1&2	2	1	2	2	
Piperales								Oxalidaceae	1	1	1	2	2	2	1	1	1	47.6
								Tremandraceae	1	1	2	2	2	2	1	1	1	
	1	1	1	1&2	1		2	Piperaceae	1	1	1	1&2	1	1	2	2		
	1	1	1	2	2		5	Myrsinaceae	1	1	1	2	2	2	3	5		
	1	1	1&2	1	1	1	6	Menispermaceae	1	1	1	1	1	1	1	2	15.3	
								Papaveraceae	1	1	1&2	1	1	1	2	3		
								Ranunculaceae	1	1	2				1	1		
								Rhamnaceae	1	1&2	1	1&2	1	1	1	5	8	27.5
								Loranthaceae	1&2	1	2	2	2	1	2	8	8	
								Oliaceae	1&2	1&2	1				2	2		
Sapindales								Santalaceae	1	1	1&2				2	3		
								Anacardiaceae	1	1	2	2	1	1&2	6	7	31.2	
								Buseraceae	1	1	2	2	2	2	2	5		
								Meliaceae	1&2	1&2	1&2	1&2	1	1&2	12	32	57.7	
								Rutaceae	1&2	1	1&2	1&2	1&2	1&2	21	54	37.7	
								Sapindaceae	1&2	1&2	1&2	1&2	1	1&2	23	65	38.6	
								Simaroubaceae	1&2	2	1&2				2	4		

continued

APPENDIX 1—*continued*

Order	germ	leaf	node	hypo	coty	emb	<i>n</i> ₁	Family	germ	leaf	node	hypo	coty	emb	<i>n</i> ₂	<i>n</i> ₃	oil
Solanales	1	1	1	1&2	1&2	1&2	29	Boraginaceae	1	1	1	2	2	2	2	4	
								Convolvulaceae	1	1	1	1	1	2	11	22	12.3
								Solanaceae	1						2	3	
Urticales	1&2	1&2	1&2	1&2	1&2		51	Moraceae	1&2	1&2	1&2	1&2	1	1	5	36	
								Ulmaceae	1	1	2	2	2	2	3	8	
								Urticaceae	1	1	1&2	1&2	1&2	1&2	4	7	
Violales	1&2	1	1&2	1&2	1&2	1&2	106	Chrysobalanaceae	1						1	1	53.6
								Clusiaceae	2					1	5	13	
								Erythroxylaceae	1						1	2	
								Euphorbiaceae	1&2	1	1&2	1&2	1&2	1&2	35	76	36.8
								Flacourtiaceae	1&2					2	6	9	23.5
								Passifloraceae	1	1	2	1	1	1	1	1	
								Rhizophoraceae	1&2						2	2	
								Violaceae	1	1	1	1	1	1	2	2	
Zygophyllales	1	1	1	1	1		1	Zygophyllaceae	1	1	1	1	1	1	1	1	
(Aust/Wint)	1&2	1	1	1&2	1&2		3	Austrobaileyaceae	2	1	1	2	2	1	1	1	
								Winteraceae	1			1	1	1	1	2	
								Aquifoliaceae	1						2	2	
								Droseraceae	1&2	1	1&2	1	1	1	1	15	
								Nepenthaceae	1						1	1	
								Pumbaginaceae	1	1	1&2	1	1	1	2	2	
								Polygonaceae	1	1	1	1&2	1&2	1	2	9	2.8
								Proteaceae	1&2	1&2	1&2	1&2	1&2	1	30	126	23.4
								Crassulaceae	1	1	2	1	1	1	1	1	
								Dilleniaceae	1	1	1	1	1	1	2	4	
								Haloragaceae	1	1	2	1	1	1	2	3	
								Hamamelidaceae	1						3	3	
								Leeaceae	1	1	1	1	1	1	1	1	
								Vitaceae	1	1	1	1&2	1	1	4	11	
								Corynocarpaceae	1	1	1	1	1	1	1	1	
								Davidsoniaceae	2						1	1	
								Elacocarpaceae	1	1	1	1&2	1&2	1&2	4	25	
								Icacinaceae	1	1	1	1	1	2	5	6	
								Rosaceae	1&2	1	1	1&2	1&2	1	4	6	23.4
								Stackhousiaceae	1	2	1				1	1	

APPENDIX 2

Cross-tabulation of binary characters and log seed volume by fruit type. *P* values for binary characters from contingency test; for log seed volume, *P* value from ANOVA.

trait	state	achene	berry	capsule	cypsela	drupe	legume	nut	schizocarp	utricle	<i>n</i>	<i>P</i>
germination	phanerocotylar	7	16	132	17	29	63	16		7	296	<0.001
	cryptocotylar	2	63	24		92	6				187	
first leaf	leaf-like	5	13	108	13	19	63	7		7	242	<0.001
	scale-like	1	59	11		87	5	7	1		171	
first node	leaf single	3	5	44	5	16	49	3		3	133	<0.001
	paired	3	67	73	8	8	18	10	2	3	192	
hypocotyl	glabrous	5	9	81	15	12	59	8		7	201	0.001
	hairy		4	26	2	8	5	4	2		51	
cotyledons	glabrous	4	12	84	13	14	62	13	5	7	214	0.027
	hairy		1	21	3	5	1	2	2		35	
embryo	colourless	3	9	85	14	102	19	8	4	5	249	<0.001
	green	1	6	29		4	3		1	2	46	
log seed vol	mean	-0.043	2.73	0.356	-0.297	1.568	1.180	0.338	-0.730	-0.190		<0.001
	SD	1.05	1.20	1.39	0.878	1.15	0.771	0.491	0.682	0.355		
	<i>n</i>	7	71	100	13	27	58	14	5	7		