

Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms *a priori* predictions: evidence from an *ndhF* phylogeny

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We present a well-resolved, highly inclusive phylogeny for monocots, based on *ndhF* sequence variation, and use it to test *a priori* hypotheses that net venation and vertebrate-dispersed fleshy fruits should undergo concerted convergence, representing independent but often concurrent adaptations to shaded conditions. Our data demonstrate that net venation arose at least 26 times and was lost eight times over the past 90 million years; fleshy fruits arose at least 21 times and disappeared 11 times. Both traits show a highly significant pattern of concerted convergence ($p < 10^{-9}$), arising 16 times and disappearing four times in tandem. This phenomenon appears driven by even stronger tendencies for both traits to evolve in shade and be lost in open habitats ($p < 10^{-13}$ – 10^{-29}). These patterns are among the strongest ever demonstrated for evolutionary convergence in individual traits and the predictability of evolution, and the strongest evidence yet uncovered for concerted convergence. The rate of adaptive shifts per taxon has declined exponentially over the past 90 million years, as expected when large-scale radiations fill adaptive zones.

Keywords: adaptation; correlated evolution; molecular systematics; monocotyledons

1. INTRODUCTION

Over the past decade, molecular systematics has revolutionized our understanding of higher-level relationships within the angiosperms, shifting genera among families and families among orders and leading to the first coherent view of the phylogeny of flowering plants as a whole (Chase *et al.* 1993; Soltis *et al.* 2000; APG 2003). Such analyses provide the basis for rigorous studies of adaptive radiation, historical biogeography and the evolution of

development, independent of convergence among distantly related taxa or divergence among close relatives (Givnish 1997; Soltis *et al.* 1999; Zanis *et al.* 2003; Davies *et al.* 2004).

Within the angiosperms, higher-level relationships are today perhaps best understood in the monocotyledons (Chase *et al.* 2000, 2005; Michelangeli *et al.* 2003; Davis *et al.* 2004; Graham *et al.* 2005). Monocots—with approximately 60 000 species, 92 families and 12 orders—are by far the most species-rich, morphologically diverse and ecologically successful of the early-divergent¹ clades of angiosperms, from which—based

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on molecular data (Bremer 2000)—they appear to have diverged more than 160 Myr ago. Cladistic analysis of more than 500 sequences of *rbcL* plastid DNA sequences identified six major monocot clades: the commelinids (including the orders Poales, Commelinales, Zingiberales, Dasypogonales and Arecales), Asparagales, Liliales, Pandanales, Dioscoreales and Alismatales, with *Acorus* being sister to all other monocots (Chase *et al.* 1995). Yet, even when these data were augmented with sequences for plastid *atpB* and nuclear ribosomal 18S for a smaller subset of 140 species (Chase *et al.* 2000), relationships among most major clades remained unresolved or weakly supported and relationships among several lineages of commelinids and asparagoids remained poorly understood.

To investigate the intriguing phenomena of concerted convergence and plesiomorphy (see below), we produced a well-resolved, highly inclusive monocot phylogeny based on *ndhF* sequence variation (figure 1). Plastid-encoded *ndhF* provides a wealth of data for phylogenetic reconstruction: it is a larger gene (*ca.* 2200 bp) than *rbcL* (*ca.* 1464 bp) and has a greater fraction of variable sites for a given set of taxa (Gaut *et al.* 1997; Patterson & Givnish 2002). Our *ndhF* phylogeny provides the best available basis for analysing patterns of repeated convergence and divergence within the monocots, involving many more characters, greater resolution and better support for specific clades than *rbcL* alone (albeit for fewer taxa), and including many more lineages than the existing 3-gene phylogeny based on *rbcL*, *atpB* and 18S (Chase *et al.* 2000), or the 7- and 17-gene trees now in press (Chase *et al.* in press; Graham *et al.* in press). The latter are about as well resolved as our *ndhF* phylogeny, albeit better supported.

Concerted convergence (Givnish & Sytsma 1997) is the independent rise of two or more traits that are genetically, developmentally and functionally unrelated in different lineages under similar ecological conditions; concerted plesiomorphy involves the evolutionary retention of the same suite of traits in different lineages under similar conditions (Patterson & Givnish 2002). Both phenomena are examples of correlated evolution and can result from the adaptation of unrelated traits to the same environmental conditions or different components of the same *set* of conditions. Both may be especially challenging to detect and study using phylogenies based on traditional phenotypic data, given that multiple (and seemingly independent) characters would carry the same, misleading signal regarding evolutionary relationships. Within monocots, Patterson & Givnish (2002) demonstrated that concerted convergence and plesiomorphy occur in the order Liliales. Phylogenetic reconstruction showed that (i) visually showy flowers, capsular fruits, wind-dispersed seeds, narrow leaves, parallel venation and bulbs evolved upon invasion of open seasonal habitats and (ii) visually inconspicuous flowers, fleshy fruits, animal-dispersed seeds, broad thin leaves, net venation and rhizomes were retained in lineages inhabiting ancestral forest habitats. For each trait, the observed variation in phenotype with environment across lineages appears to be functionally adaptive.

(a) *Predicted patterns of concerted convergence*

Some of these patterns of concerted convergence and plesiomorphy may hold throughout the monocots. In this paper, we test the hypotheses that net venation and vertebrate-dispersed fleshy fruits frequently evolve and are retained with each other and life in shaded forest understories, and that parallel venation and non-fleshy fruits (dispersed mainly by wind, water or gravity) frequently evolve and are retained with each other and life in open habitats.

The functional rationale for these predictions is as follows. First, even though parallel veins are a monocot hallmark, shady conditions should favour the evolution of net (i.e. branching) venation based on biomechanical economy (Givnish 1979). Shady conditions favour thin, broad leaves, which cannot support themselves mechanically (especially after small losses in turgor pressure) and thus require longitudinal and lateral reinforcement from pronounced primary and secondary veins. Because the cost per unit length of such veins scales like their diameter squared, while their strength scales like their diameter cubed, the economics of support in soft, thin, broad leaves favours the coalescence of nearby, nearly parallel veins into one or a few branching ribs of lower total cost. The broader and thinner a leaf or its divisions, the greater should be the advantage of net venation and a single midrib. Here we add that if soft, thin, broad leaves are favoured by conditions other than shade—specifically, in fast-growing, emergent aquatic plants with access to abundant moisture and nutrients (e.g. *Sagittaria*) and in filmy-leaved submersed species adapted for photosynthesis underwater (e.g. *Aponogeton*)—net venation should also evolve under those conditions. Givnish (1979) observed that net venation occurs in several monocot groups with thin, broad leaves in forest understories, including *Arisaema*, *Smilax*, *Trillium* and various tropical ginger. Conover (1983) and Chase *et al.* (1995) noted similar, qualitative associations of net venation with broad-leaved forest vines; Cameron & Dickison (1998) later noted an association with achlorophyllous vanilloid orchids.

Second, wind dispersal of seeds is likely to succeed in open, windy habitats, but animal dispersal of fleshy fruits should be more effective below closed canopies (Croat 1978). Indeed, up to 95% of the woody understory species in neotropical rain forests bear fleshy fruits (Gentry 1982). Phylogenetic reconstructions indicate that fleshy fruits have evolved repeatedly in association with forest understories in commelinids (Givnish *et al.* 1999) and Liliales (Patterson & Givnish 2002) among monocots, and in Lobeliaceae (Givnish 1999), Gesneriaceae (Smith 2001) and Urticales (Sytsma *et al.* 2002) among dicots.

2. MATERIAL AND METHODS

To test our hypotheses, we derived a monocot-wide phylogeny based on *ndhF* sequences for 282 species chosen to represent as broad a swath of the monocots as possible, including members of 78 of 92 families and all 12 orders (see Electronic Appendix). The unsampled families are generally very small and account for only 1.2% of monocot species. The data were analysed using maximum parsimony, with *Ceratophyllum* as the out-group based on its sister position

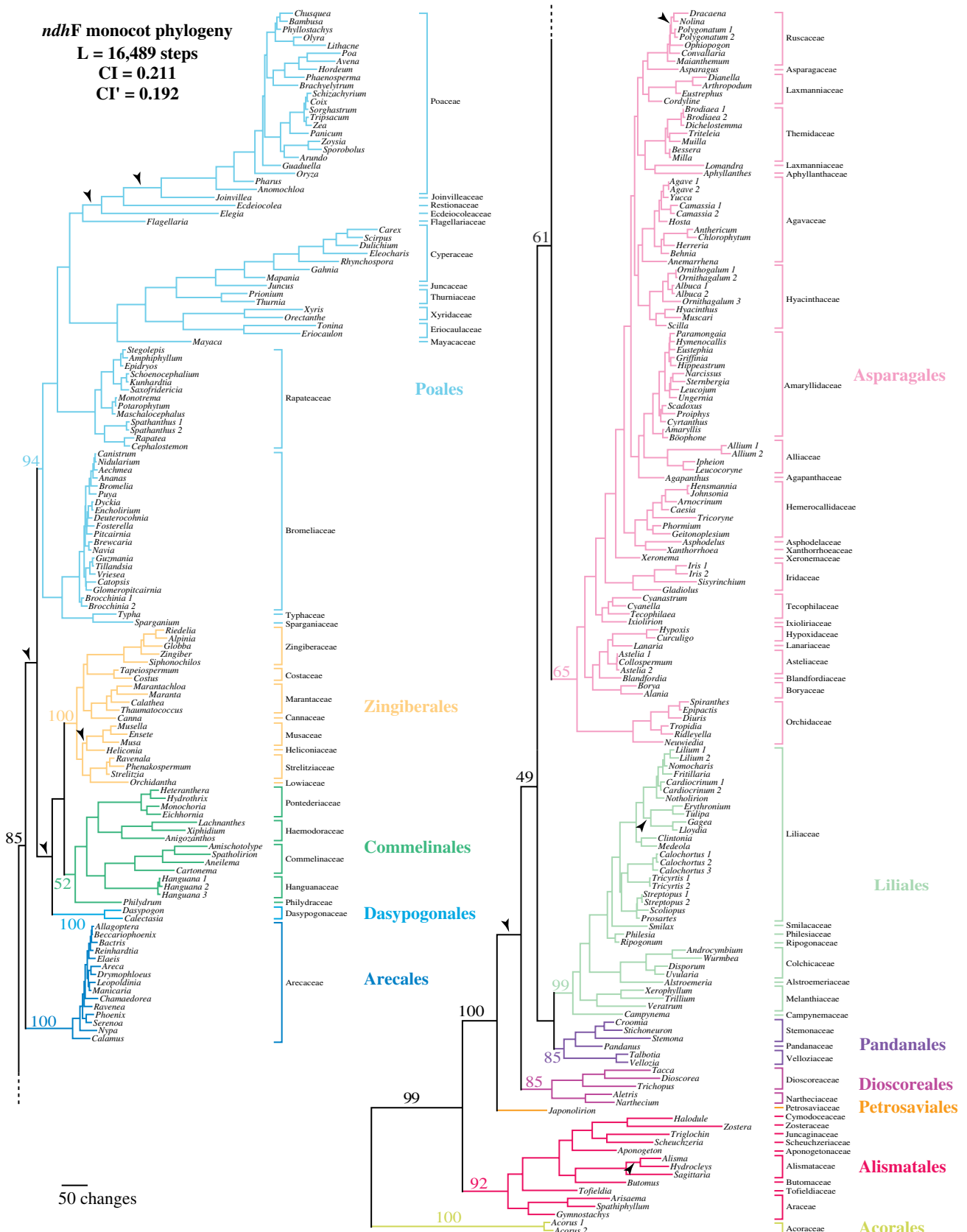


Figure 1. A phylogram of one of 874 most-parsimonious trees produced by a maximum-parsimony analysis of *ndhF* sequence variation. Branch lengths are proportional to the number of mutations inferred down each lineage. Arrowheads indicate nodes that collapse in the strict consensus tree; bootstrap values (orders and commelinids only) are indicated above each node. See Electronic Appendix for bootstrap values at other nodes.

to the monocots in recent studies (Chase *et al.* 2000; Soltis *et al.* 2000).

We tested whether fleshy fruits, net venation and occurrence in shaded forest understories show

correlated evolution using DISCRETE (Pagel 1994). DISCRETE employs a continuous Markov model to examine the evolution of binary characters, taking branch length into account and weighting gains and losses equally.

We conducted separate tests of correlated evolution between (i) fleshy fruits and shady habitats, (ii) net venation and shady habitats, (iii) fleshy fruits and net venation, (iv) net venation and shady habitats, emergent broad-leaved aquatics or submersed broad-leaved aquatics and (v) fleshy fruits and net venation, excluding emergent and submersed broad-leaved aquatics (see Electronic Appendix). We ran each test on four fully resolved trees, chosen at random from each of the four resolutions of the polytomy at the base of the commelinids. The other unresolved nodes in the strict consensus tree appear to have few, if any, effects on inferences regarding the evolution of fleshy fruits or net venation.

To calculate the timing of the origins of fleshy fruits and net venation, we transformed one of the most-parsimonious trees into ultrametric form using cross-verified penalized likelihood (PL), calibrating the tree against the ages of six Cretaceous fossils and the inferred divergence of *Acorus* from other monocots 134 Myr ago (see Electronic Appendix). Penalized likelihoods (Sanderson 2002) average local differences in the rate of DNA evolution on different branches, taking into account branch lengths and branching topology. PL differs from non-parametric rate smoothing (NPRS; Sanderson 1997) in assigning a penalty for rate changes among branches that are too rapid or frequent, based on a smoothness parameter. If the smoothness parameter is large, then PL approaches NPRS. NPRS behaves well in trees with substantial rate variation, but suffers when rates are clock-like or nearly so (Sanderson 2002, personal communication). We employed the cross-verification algorithm in *r8s* to find the optimal value of the smoothness parameter, minimizing the sum of the squared deviations between observed and expected branch lengths, derived through jack-knifing each individual branch (Sanderson 2002). The smoothness parameter was varied from 10^0 to 10^3 by steps of 0.25 in the exponent.

We overlaid net venation, fleshy fruits and life in shady habitats on this phylogeny using accelerated transformation (to minimize the number of parallel gains inferred), tabulated the number of origins of each character every 10 Myr and then normalized these rates against the number of clades present at the midpoint of each 10 Myr period. For our purposes, 'net venation' includes all leaves with branching support networks, including (i) reticulate venation, (ii) simple leaves in which the veins diverge from a massive central rib regardless of whether they branch anatomically (e.g. *Costus*, *Musa*) and (iii) compound leaves with branching rachis (palms). Occasional cross-veins in leaves with otherwise rigorously parallel venation, with multiple major veins, were considered instances of 'parallel venation', given that such a pattern is essentially equivalent to a purely parallel venation on biomechanical grounds and is inconsistent with the expected biomechanical optimization on to a strongly branching network (see above). 'Fleshy fruits' include berries, drupes and seeds with showy and massive arils dispersed by vertebrates, all with moist, sweet (or oily) tissue. Brightly coloured seeds or capsules that mimic fleshy fruits (*Griffinia*, *Hippeastrum*, *Proiphys*) were scored as such. Seeds dispersed by ants, bearing small arils (elaiosomes), occur in forest and non-forest plants and can serve as adaptations not directly related to dispersal (e.g. emplacement in nutrient-rich ant nests or shelter from frequent fire; Beattie 1985). The fruits of *Acorus* are berries but have dry coats and lack the sweet or oily composition usually associated with vertebrate dispersal—that is, they are

anatomically but not functionally fleshy fruits. Other features of *Acorus* morphology and its geographical pattern of genetic variation strongly implicate water dispersal (Liao & Hsiao 1998). Consequently, neither ant-dispersed seeds nor *Acorus* berries were scored as fleshy fruits. Finally, species were categorized based on their occurrence in primarily open sunny habitats (e.g. tundra, grasslands or chaparral) or primarily closed shady habitats (forest under-stories). For deciduous forests, the timing of leaf activity and fruit production relative to canopy closure was used to classify the habitats occupied (see Electronic Appendix).

3. RESULTS

(a) *ndhF* phylogeny

Maximum parsimony produces one island of 874 trees, each 16 489 steps in length based on 1727 variable characters, of which 1408 are phylogenetically informative (figure 1). Across monocots, *ndhF* supports each of the 12 orders identified by prior molecular research (65–99% bootstrap values, excluding Commelinales) and clarifies several nodes that were previously unresolved or weakly supported. Our data show that (i) Asparagales is sister to the commelinids, (ii) both groups are sister to Liliales and Pandanales, (iii) *Japonolirion* (Petrosaviales), then Dioscoreales are sister to all preceding groups and (iv) Alismatales is sister to the strongly supported (100% bootstrap) clade of the preceding orders, followed by *Acorus* (Acorales). Bootstrap support based on *ndhF* exceeds or equals that based on *rbcL*, 18S and *atpB* for 10 of the 12 nodes at or above the ordinal level reported by Chase *et al.* (2000). Nine nodes are unresolved in the strict consensus. Of these, only two—involving a four-way polytomy at the base of the commelinids and a trichotomy involving four families of Zingiberales—involve substantial numbers of species (figure 1). Our analysis strongly supports each of four major commelinid clades (Poales, Commelinales, plus Zingiberales, Arecales and Dasypogonales). The lack of resolution of relationships among these clades may simply reflect a rapid initial diversification of commelinids; analyses based on 17 genes (but many fewer taxa) also fail to conclusively resolve this node (Graham *et al.* in press). Among the most-parsimonious *ndhF* trees, four different relationships among the major commelinid clades emerge: ((Poales, Arecales), (Commelinales–Zingiberales, Dasypogonales)); (Poales, (Commelinales–Zingiberales, Arecales, Dasypogonales)); ((Poales, (Commelinales–Zingiberales, Dasypogonales)), Arecales) and ((Poales, Dasypogonales), Arecales), (Commelinales–Zingiberales). A detailed description of the systematic implications of our *ndhF* phylogeny will be presented elsewhere.

(b) *Concerted convergence*

Based on our *ndhF* phylogeny, fleshy fruits appear to have arisen at least 21 times and been lost 11 times, while net venation has arisen at least 26 times and been lost 8 times (table 1, figure 2). As predicted, these traits have undergone repeated concerted convergence. They have done so in highly significant fashion ($p < 10^{-9}$, log-likelihood test), with both traits arising together 16 times and disappearing together four times (tables 1 and 2, figure 2). Fleshy fruits and net venation arose together in Joinvilleaceae, Flagellariaceae, Hanguanaceae, Arecales,

Table 1. Evolutionary origins of net venation, fleshy fruits and life in shady microsites, and of parallel venation, passively dispersed seeds and life in sunny microsites. (Most origins of the former character-states represent initial transitions from the latter, while most origins of the latter represent reversals from the former. Transitions on the same line occurred at the same or (in a few cases) adjacent nodes. Taxa in which all three character-states underwent transition at the same or adjacent nodes—entailing concerted convergence—are indicated in bold. All calls are based on overlaying characters on a single most-parsimonious tree using accelerated transformation (see Electronic Appendix).)

net venation	fleshy fruits	shade
Bambusoideae <i>Joinvillea</i> + basal Poaceae <i>Flagellaria</i>	<i>Joinvillea</i> <i>Flagellaria</i>	Bambusoideae <i>Joinvillea</i> + basal Poaceae <i>Flagellaria</i>
Hanguanaceae	Bromelioideae Hanguanaceae <i>Amischolotype</i>	Monotremeae Bromelioideae – (Hanguanaceae + Commelinaceae–)
Zingiberales + Philydraceae^a Areaceae <i>Hosta</i> <i>Behnia</i>	Zingiberales Areaceae <i>Behnia</i>	Zingiberales Areaceae <i>Hosta</i> <i>Behnia</i> <i>Chlorophytum</i>
<i>Polygonatum</i> basal Ruscaceae + <i>Griffinia</i> <i>Hymenocallis</i> <i>Proiphys</i> + <i>Scadoxus</i> <i>Geitonoplesium</i> <i>Cyanastrum</i> <i>Curculigo</i>	<i>Polygonatum</i> <i>Griffinia</i> + <i>Hippeastrum</i> <i>Proiphys</i> + <i>Scadoxus</i> <i>Geitonoplesium</i> <i>Curculigo</i> Asteliaceae + Blandfordiaceae <i>Neuwiedia</i>	Ruscaceae + Laxmanniaceae Laxmanniaceae <i>Griffinia</i> + <i>Hippeastrum</i> <i>Hymenocallis</i> <i>Proiphys</i> + <i>Scadoxus</i> <i>Geitonoplesium</i> <i>Cyanastrum</i> <i>Curculigo</i> Asteliaceae + Blandfordiaceae <i>Neuwiedia</i> <i>Epipactis</i> <i>Tropidia</i> <i>Cardiocrinum</i>
<i>Cardiocrinum</i> higher Liliales (> <i>Alstroemeria</i>)	<i>Cardiocrinum</i>^b higher Liliales (> <i>Ripogonum</i>)	higher Liliales (> <i>Ripogonum</i>) <i>Calochortus albus</i> <i>Disporum</i> – <i>Uvularia</i> <i>Trillium</i>
<i>Disporum</i> – <i>Uvularia</i> <i>Trillium</i> Stemonaceae	<i>Disporum</i> <i>Trillium</i>	Stemonaceae
Dioscoreaceae Alismataceae ^a <i>Zostera</i>^a <i>Aponogeton</i>^a Araceae	Pandanaceae <i>Tacca</i> Araceae	Dioscoreaceae Alismataceae ^a Araceae
parallel venation	passively dispersed fruits	sun
higher Poaceae	Poaceae	higher Poaceae <i>Cartonema</i>
	Costaceae Cannaceae ^a	Cannaceae Strelitziaceae
	Lowiaceae <i>Nypa</i>	<i>Nypa</i> <i>Phoenix</i>
<i>Dracaena</i> + <i>Nolina</i>	<i>Nolina</i> <i>Asparagus</i>	<i>Dracaena</i> + <i>Nolina</i> <i>Asparagus</i>
<i>Arthropodium</i> <i>Hypoxis</i> – <i>Lanaria</i> Lilioideae <i>Calochortus</i>	<i>Arthropodium</i> <i>Hypoxis</i> – <i>Lanaria</i> Lilioideae <i>Calochortus</i> + <i>Tricyrtis</i>^b <i>Scoliopus</i>	<i>Arthropodium</i> <i>Hypoxis</i> – <i>Lanaria</i> Lilioideae <i>Calochortus</i>
<i>Androcymbium</i> – <i>Wurmbea</i>		

^a Associated with broad-leaved aquatic habit.

^b Associated with origin or retention of passively dispersed fruits adapted to dispersal in autumn under open canopies in deciduous forests, with net venation adapted for leaf activity in summer under closed canopies.

Zingiberales, *Behnia*, two groups of Amaryllidaceae, *Geitonoplesium*, *Curculigo*, the core Liliales, *Trillium* and Araceae, and are associated with each other in *Tacca*, *Disporum* and *Polygonatum*, with the evolution of fleshy

fruits slightly lagging that of net venation among close relatives and inferred ancestors in the latter three lines (figure 2). Fleshy fruits and net venation were lost together in *Arthropodium*, *Hypoxis*–*Lanaria*, Lilioideae and *Nolina*,

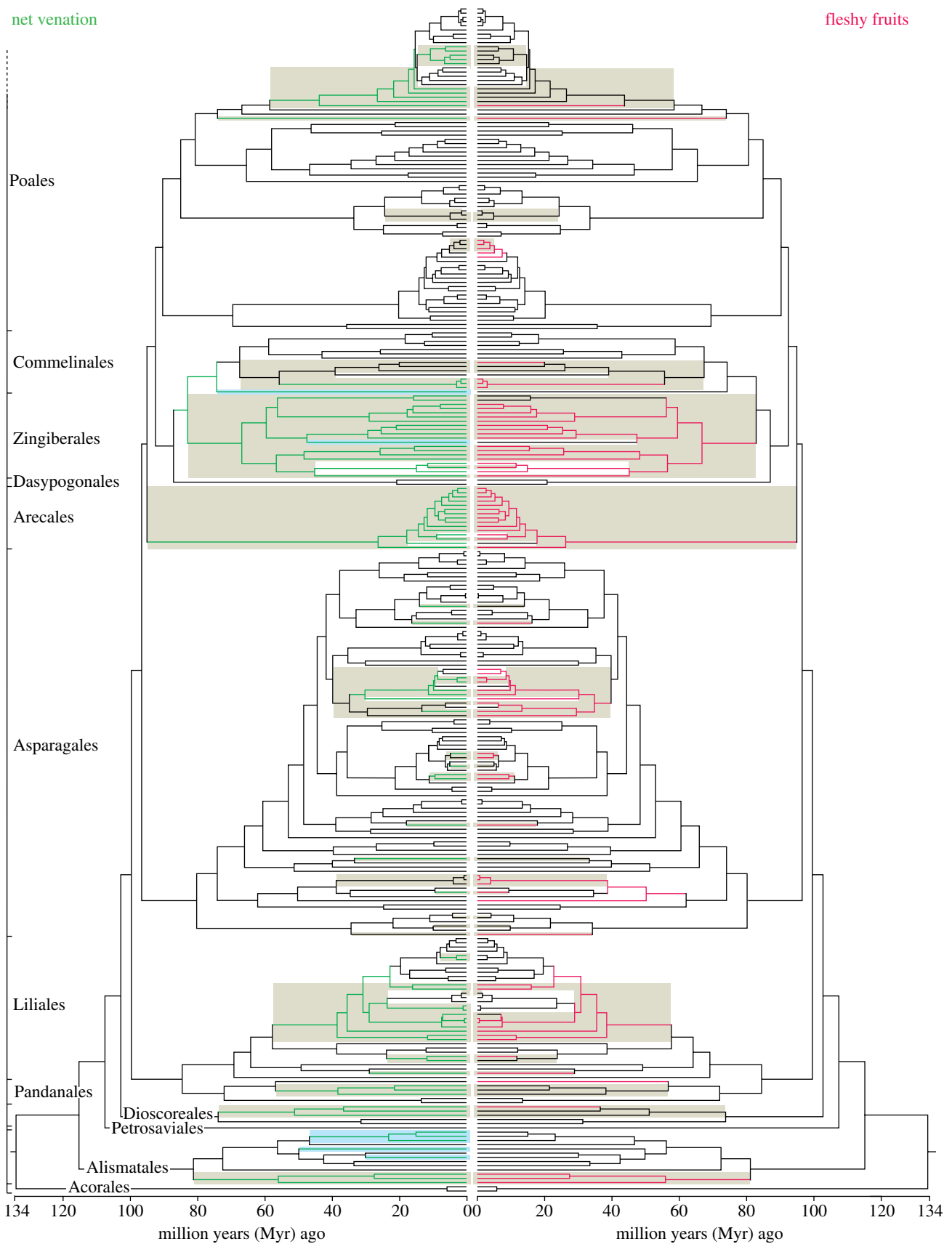


Figure 2. Concerted convergence of net venation (green), fleshy fruits (red), shaded habitats (sand boxes) and broad-leaved aquatic habitats (blue boxes). Note that almost all transitions to net venation and fleshy fruits occur upon invasion of shaded habitats, and that almost all reversals to parallel venation and dry, passively dispersed seeds or fruits occur upon re-invasion of open, sunny habitats. Branching has been calibrated against time based on fossil data, so that the tempo and taxonomic distribution of phenotypic transitions can be visualized.

Table 2. Log-likelihood ratios (LR) and significance levels from five tests of correlated evolution across monocots, involving net venation, fleshy fruits, life under shaded conditions and their converses. (Mean \pm s.e. log-likelihood ratios reflect the outcome of five analyses per test per tree; grand standard errors are based on the tree-based means, minimums and standard errors displayed).

	tree A	tree B	tree C	tree D	mean	grand s.e.	significance
<i>fleshy fruits and net venation</i>							
mean LR	53.0	50.1	53.0	51.2	51.8	0.7	
s.e.	0.5	0.5	0.8	0.6	0.7	0.08	
minimum	51.4	48.6	51.3	49.8	50.3	0.7	$p < 10^{-9}$
<i>fleshy fruits and shade</i>							
mean LR	70.8	70.8	72.0	71.0	71.1	0.3	
s.e.	0.6	0.4	0.5	0.6	0.5	0.05	
minimum	68.9	69.8	70.7	69.4	69.7	0.4	$p < 10^{-13}$
<i>net venation and shade</i>							
mean LR	115.2	114.6	116.0	115.4	115.3	0.3	
s.e.	0.5	0.4	0.5	0.6	0.5	0.05	
minimum	113.8	114.0	115.1	113.6	114.1	0.4	$p < 10^{-22}$
<i>net venation and shade + broad-leaved aquatics</i>							
mean LR	146.4	144.3	146.4	146.2	145.8	0.5	
s.e.	0.4	0.9	0.3	1.1	2.5	0.19	
minimum	145.7	141.2	145.4	142.6	143.7	1.1	$p < 10^{-29}$
<i>fleshy fruits and net venation, excluding broad-leaved aquatics</i>							
mean LR	63.5	64.7	63.8	60.4	63.1	0.9	
s.e.	0.4	0.4	0.8	1.9	0.9	0.36	
minimum	62.3	63.9	60.9	56.7	61.0	1.6	$p < 10^{-10}$

with the loss of fleshy fruits in the latter lagging that of net venation by one node. These conclusions would be unaltered if *Acorus* were instead scored as having fleshy fruits.

Both fleshy fruits and (especially) net venation show even stronger patterns of correlated evolution with shady conditions. In almost every case, the evolution of net venation and fleshy fruits is associated with life in forest understories, while their loss is associated with open habitats. Specifically, 19 of 21 gains of fleshy fruits are associated with life in shady sites, while eight of 11 losses are associated with life in sunny sites (figure 2). For net venation, 22 of 26 gains are associated with shady conditions and all eight losses are associated with sunny conditions. These patterns of origin and maintenance are highly significant ($p < 10^{-13}$ – 10^{-22}) when tested in DISCRETE (table 2). These results support our adaptive hypotheses and establish the existence of an extraordinary, highly significant pattern of concerted convergence across the monocots.

Net venation shows an even stronger association with shade ($p < 10^{-29}$) if we factor out the four lineages (Alismataceae, Aponogetonaceae, Philydraceae and Zosteraceae) in which it arose in broad-leaved aquatic plants, mostly near the base of the monocots in Alismatales (figure 2). All origins of net venation are associated with shady conditions or broad-leaved emergent or submersed aquatics (tables 1 and 2). As expected, fleshy fruits show a stronger association with net venation if we exclude net-veined, broad-leaved aquatic plants, for which we have no *a priori* reason to expect the evolution of fleshy fruits (table 2).

The numerous origins of fleshy fruits and net venation are distributed rather evenly across lineages and time (figure 2). Both traits first arose 80–90 Myr ago in Areaceae and Araceae. Palms include many rainforest

groups but have also invaded open savannas. Aroids are mainly herbs, vines and epiphytes of densely shaded tropical rain forests, together with some temperate forest herbs (e.g. *Arisaema*) and broad-leaved submersed aquatics (e.g. *Anubias* and *Cryptocoryne*). The most recent instances of concerted convergence in fleshy fruits and net venation occurred in *Curculigo* of Hypoxidaceae within the last 5 Myr. Fleshy fruits evolved at least three times in Poales, twice in Commelinales, once in Zingiberales, once in Arecales, eight times in Asparagales, three times in Liliales, once in Pandanales, once in Dioscoreales and once in Alismatales. Net venation evolved at least three times in Poales, once in Commelinales, once in Zingiberales, once in Arecales, ten times in Asparagales, four times in Liliales, once in Pandanales, once in Dioscoreales and four times in Alismatales (figure 2). The frequency of adaptive change (both gains and losses) in both characters per clade declines exponentially with time (figure 3), as expected—but rarely documented—for adaptive radiations as they ‘fill’ ecological space.

Only net venation evolved in some understory groups, including the bambusoids, basal grasses, Costaceae, *Hosta*, Tecophilaeaceae and Stemonaceae. *Cardiocrinum* and *Tricyrtis* of temperate deciduous forests both have net veins only, but are photosynthetically active under shady conditions in summer, releasing seeds after the canopy re-opens in autumn. Net veins also occur in the absence of fleshy fruits in four lineages of broad-leaved aquatics. Fleshy fruits arose without net venation under shady conditions in the bromelioid bromeliads, *Amischotolype*, Asteliaceae and relatives, and the apostasioid orchid, *Neuwiedia*.

4. DISCUSSION

In many ways, the contrast between *Trillium* and its closest relatives in Melanthiaceae (represented by *Xerophyllum*)

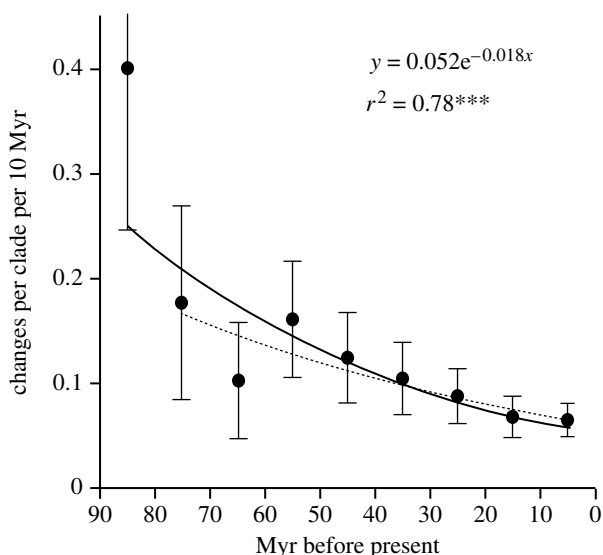


Figure 3. Rate of adaptive transitions in venation and fruit characters (gains and losses per clade per 10 Myr) in the monocots over the past 90 Myr. The rate of such transitions was highest early in monocot evolution and subsequently dropped exponentially with time ($y=0.052e^{-0.018x}$; $r^2=0.78$, $p<0.001$ for 7 d.f.). Dashed curve represents the regression without data for the earliest interval (outlier for mean, variance) included. This analysis underestimates the decline in the rate of adaptive change, because our sample (ca. 0.5% of all monocot species) is biased towards including families and groups in which net venation and fleshy fruits have evolved, and away from the most recent, phenotypically repetitive diversification within families and genera. Inclusion of the latter would greatly inflate the number of recent clades and add few new shifts in venation or fruit type.

crystallizes the pattern of concerted convergence discussed here. *Trillium* of forest understories has broad, thin, soft leaves, net venation and berry-like capsules, while *Xerophyllum* usually grows in more open habitats and possesses narrow, thick, hard leaves, parallel venation and tiny, wind-dispersed seeds. It would be difficult, based on gross morphology, to infer that these taxa are, in fact, very close relatives.

Across the monocots surveyed, mode of seed dispersal and leaf venation pattern show a phylogenetically uncorrected correlation (r) of 0.58. Mode of seed dispersal and light availability (sun versus shade) show a stronger correlation ($r=0.66$), and leaf venation and light availability are even more strongly correlated ($r=0.74$). The last value rises to 0.79 if we include the origin of net venation in broad-leaved aquatic plants (see above). The evolution of fleshy fruits and net venation is not lock-step: by no means is every invasion of forest understories associated with a gain of both traits, nor is every invasion of open sites associated with a loss of both traits. Nevertheless, the patterns seen are highly significant and explanatory and some of the apparent exceptions are illuminating. Bromelioid bromeliads evolved fleshy fruits but not net venation—perhaps understandable, given that they possess Crassulacean acid metabolism photosynthesis and thick, succulent leaves. Vanilloid orchids (not included in our survey) evolved net venation but not fleshy fruits (except in *Vanilla* itself)—also understandable, given that mycotrophy favours tiny, numerous, independently dispersible seeds to maximize

the chances of contacting a suitable fungal partner. Some discords, like the absence of fleshy fruits in the net-veined basal grasses and bambusoids, remain puzzling. The persistence of net venation and fleshy fruits in some groups of open habitats, notably *Ravenala* and *Strelitzia* of Zingiberales, appear to be best explained by phylogenetic inertia.

Beyond the taxa surveyed, a few additional cases may represent joint origins of fleshy fruits and net venation under shady conditions. Examples include *Vanilla* (Orchidaceae), *Palisota* (Commelinaceae), *Eucharis* (Amaryllidaceae) and Cyclanthaceae of tropical rainforest understories. However, one of the most striking cases may involve the tropical vine *Gnetum*, which has fleshy fruits and broad, net-veined leaves that strongly resemble those of *Coffea* and other understory angiosperms—despite the fact that it is a gymnosperm! The strong resemblance of *Gnetum* to certain angiosperms inspired the hypothesis that angiosperms were derived from Gnetales (Doyle & Donoghue 1986); molecular data have largely laid that hypothesis to rest, placing gymnosperms sister to angiosperms and indicating that Gnetales is well-nested within the conifers (Chaw *et al.* 2000; Soltis *et al.* 2002; but see Rydin *et al.* 2002).

The fact that fleshy fruits and net venation have each arisen more than 20 times in the monocotyledons raises the key question of whether the same developmental pathways and underlying genes were involved in each case, or whether these adaptations arose in different ways in different groups. It seems unlikely that more than 20 origins of fleshy fruits and net venation over 90 Myr all tapped into the same genes and developmental pathways, and several lineages show obvious differences in the fine details of venation pattern or use different tissues to attract dispersers. Determining the genetic and developmental bases for these shifts should be a major goal of new studies at the interface of ecology, evolution and development ('eco-evo-devo' (Givnish 2003)). An especially promising dicot group to investigate in this regard might be the Hawaiian silversword alliance, a recently derived (<6 Myr ago) dicot group with a relationship between leaf thickness and venation pattern similar to that seen across monocots. Parallel venation occurs in *Argyroxiphium*, *Wilkesia* and species of *Dubautia* with narrow, thick leaves adapted to dry or extremely wet, boggy conditions, while net venation occurs in *Dubautia* species (especially *D. latifolia*) with broad, thin leaves adapted to moist, shady conditions; crosses can be made among many species in this complex (Carlquist *et al.* 2003).

5. CONCLUSIONS

The repeated, coupled evolution of net venation and fleshy fruits by monocots in forest understories, and the loss of these traits in open sites, is one of the strongest patterns ever demonstrated for the evolutionary convergence of individual traits, and the strongest evidence yet for concerted convergence. The stronger associations of net venation and fleshy fruits to shade than to each other suggests that each is primarily an independent adaptation to light regime (and its correlates) than a co-adaptation to the other trait. This finding adds to a growing set of studies demonstrating the repeatability—and indeed, the predictability—of evolution, including the repeated evolution of

the same ecomorphs of *Anolis* lizards on different islands of the Greater Antilles (Losos *et al.* 1998), the same strains of *Pseudomonas fluorescens* adapted to different microsites within laboratory beakers (Rainey & Travisano 1998), the same range of flower tube lengths and elevational distributions in *Cyanea* on different Hawaiian islands (Givnish 1999), the same benthic and limnetic forms of sticklebacks within glacial lakes (Rundle *et al.* 2000) and the same shifts in the expression of 59 genes in replicate isolates of *Escherichia coli* grown under glucose-limited conditions (Cooper *et al.* 2003). Our findings extend this general pattern for the first time to one predicted *a priori*, encompassing a 90-Myr pattern of concerted convergence in the largest clade (*ca.* 60 000 species) studied in this fashion to date. The fact that both net venation and fleshy fruits evolved more than 20 times in the monocots—and especially, that adaptive shifts in venation pattern occurred with habitat shifts in at least 34 of 44 instances (see table 1 and §4)—supports the call to re-evaluate the importance of phylogenetic constraints and the supposed unpredictability of evolution (Gould 1989).

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ENDNOTES

¹We use 'early-divergent' to refer to any clade that emerges from a deep node AND has substantially fewer extant species than its sister clade. This definition, taking into account branching topology and diversity, is unambiguous. Clearly, early-divergent clades are coeval with their sister clades and there is no implication that present-day taxa of early-divergent lineages are themselves ancient. Monocots are by far the most diverse, early-divergent clade that branched off from the main line of angiosperms before the core eudicots, which constitute the majority of flowering plants.

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