



Seed mass and the evolution of fleshy fruits in angiosperms

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Fleshy fruits, like drupes and berries, have evolved many times through angiosperm history. Two hypotheses suggest that fleshy fruit evolution is related to changes in the seed mass fitness landscape. The reduced dispersal capability following from an increase in seed mass may be counterbalanced by evolution of traits mediating seed dispersal by animals, such as fleshy fruits. Alternatively, increasing availability and capabilities of frugivores promote evolution of fleshy fruits and allow an increase in seed size. Both these hypotheses predict an association between evolution of fleshy fruits and increasing seed size. We investigated patterns of fruit and seed evolution by contrasting seed mass between fleshy and non-fleshy fruited sister clades. We found a consistent association between possession of fleshy fruits and heavier seeds. The direction of fruit type change did not alter this pattern; seed mass was higher in clades where fleshy fruits evolved and lower in clades where non-fleshy fruits evolved, as compared to their sister clades. These patterns are congruent with the predictions from the two hypotheses, but other evidence is needed to distinguish between them. We emphasize the need to integrate studies of seed disperser effectiveness, seed morphology, and plant recruitment success to better understand the frugivores' role in fleshy fruit evolution.

Extant angiosperms feature a wide range of fruit and seed characteristics associated with endozoochory, animal mediated seed dispersal, where seeds are dispersed after ingestion of fruits or seeds (Ridley 1930, van der Pijl 1972, Spjut 1994). The mutualistic interaction of vertebrate seed dispersal goes back 300 million years (Tiffney 2004), and is thus not unique to angiosperms. However, in quantitative terms, angiosperm seed dispersal by vertebrates has been a pronounced and in many regions dominating interaction at least since the Early Tertiary (Tiffney 2004). For example, in some tropical forests the majority of shrubs and trees are dispersed by vertebrates, and frugivores constitute the main part of vertebrate biomass (Fleming et al. 1987). The most common adaptation to endozoochory, a fleshy fruit pulp, has evolved many times through plant history (Herrera 1989, Eriksson and Bremer 1992, Ricklefs and Renner 1994, Bolmgren and Eriksson 2005), and two general and non-exclusive mechanisms have been proposed for the evolution of fleshy fruits: (1) the fleshy pulp evolved to attract fruit-eating vertebrates who then assist in seed dispersal (Howe and Smallwood 1982, Wenny and Levey 1998); (2) the fleshy pulp evolved to reduce detrimental effects of frugivory and granivory by containing substances that repel seed predators and/or speed up gut passage (Murray et al. 1994, Cipollini and Levey 1997, Mack 2000, Tewksbury and Nabhan 2001, Tsahar et al. 2002, Filardi and Tewksbury 2005).

The evolution of fleshy fruits may also have been affected by other parts of the life cycle, especially parts of the recruitment phase (Howe 1989, Herrera et al. 1994, Schupp and Fuentes 1995, Eriksson et al. 2000, Wenny 2000a,

Wang and Smith 2002, Balcomb and Chapman 2003). For example, Alcántara and Rey (2003) found that the limited gape size of frugivores and differential recruitment success depending on seed size generate conflicting selection pressures on seed size in *Olea europaea*. Thus, to understand the potential of frugivore selection in fleshy fruit evolution, it is important to consider how frugivore fruit selection affects traits important to plant recruitment success. A link between frugivore fruit selection and plant recruitment success is suggested by previous studies showing (1) that different frugivores disperse fruits of different size (intraspecific comparisons: Wheelwright 1985, Sallabanks 1993, Jordano 1995a, Rey et al. 1997; interspecific comparisons: Mack 1993, Jordano 1995b, 2000), (2) that fruit size and seed mass are positively correlated in and among fleshy fruited taxa (Eriksson and Ehrlén 1991, Sallabanks 1993, Jordano 1995b, Bolmgren 2004, p. 30), and (3) that different frugivores disperse seeds to different habitats (Wenny and Levey 1998, Jordano and Schupp 2000). There is also a general understanding that seed mass is an important factor in plant recruitment, affecting competitive ability, germination rate, and dispersal ability (Harper et al. 1970, Jakobsson and Eriksson 2000, Leishman et al. 2000, Coomes and Grubb 2003, Mabry 2004, Moles and Westoby 2004, Bruun and Poschold 2006).

From paleoecological data it is known that there was a dramatic increase both in seed sizes and in the proportion of animal dispersed plants around the Cretaceous-Tertiary boundary (Tiffney 1984, Wing and Boucher 1998, Eriksson et al. 2000, Tiffney 2004, Eriksson 2008). These

conspicuous changes in plant reproductive features have been related to two major events in the early Tertiary: the radiation of lineages containing present-day frugivores; and the change in vegetation patterns leading to global dominance of multi-layered, tropical-like, angiosperm-dominated forests, probably mediated by climate change and a paucity of large herbivores after the demise of the dinosaurs (Wing and Tiffney 1987, Eriksson et al. 2000, Ericson et al. 2003, Janis 2003, Springer et al. 2003, Tiffney 2004). These events have been suggested as instrumental in fleshy fruit evolution, based on the following three mechanisms, or a combination of them (Wing and Tiffney 1987): (1) evolution of large seeds and fleshy fruits may have been driven by interactions with vertebrates (Tiffney 1984, 2004), (2) altered recruitment conditions in closed, shaded, vegetation, favoring large seeds and dispersal by fleshy fruits (Eriksson et al. 2000), or (3) by simultaneous evolution of large plant life forms (Moles et al. 2005a). However, phylogenetically independent origins of angiosperm fleshy fruits are not confined to the early Tertiary, but are recurrent during the last 80 million years (Bolmgren and Eriksson 2005). Therefore, a general explanation of fleshy fruit evolution must consider time-independent mechanisms, not just referring to changes in the fauna and vegetation during the Early Tertiary.

Within extant plant communities, larger seeds are generally animal dispersed (Hughes et al. 1994) and fleshy fruited plants generally grow in tree- and shrub-dominated habitats (Willson et al. 1990, Guitián and Sanchez 1992, Herrera 2002a, Patterson and Givnish 2002, Bolmgren and Eriksson 2005). Moles et al. (2005a) refined this notion by finding that animal dispersal and larger seed mass are associated also when the phylogenetic context is considered. Evolving larger seeds implies that dispersal is reduced due to a lower number of heavier falling disseminules, a tradeoff that could explain the advantage of evolving assisted dispersal in these environments. An alternative hypothesis is that fleshy fruits evolved to circumvent the problems associated with understory recruitment conditions in closed vegetation, and thereby reduce the selection pressure for larger seeds, by using animals for more directed dispersal to canopy gaps and forest margins (Wenny 2000a).

Here, we examined seed mass between fleshy versus non-fleshy fruited sister clades in a set of phylogenetically independent matched pairs. The data also allowed us to explore whether the direction of fruit type shifts had any effect on seed mass differences between the fruit types. This is particularly interesting as seed mass distributions often overlap extensively between fruit types (Hughes et al. 1994, Leishman et al. 2000, Coomes and Grubb 2003, Moles et al. 2005a) indicating that seed mass change is not a prerequisite for fruit type and/or dispersal mode change. The specific questions asked were: 1) is there a phylogenetically consistent difference in seed mass between fleshy versus non-fleshy fruit types? 2) Are fruit type dependent differences in seed mass affected by the direction of fruit type evolution? We discuss the results in relation to the different hypotheses suggested for the evolution of fleshy fruits, as mentioned above, and in relation to three different general hypotheses for seed dispersal – ‘colonization’, ‘directed dispersal’, and ‘escape’ (Howe and Smallwood 1982).

Methods

Data set

Phylogenetically independent matched pairs (PhyMPs) consisting of one sister clade with fleshy fruit types and one sister clade with non-fleshy fruit types were used for the analyses (Felsenstein 2004). This way of sampling fruit type contrasts ignores Linnean taxonomic hierarchies. Instead, it is the phylogenetic hypotheses and the phylogenetic node of fruit type change that defines the sister clades (and the taxa included in them) to be contrasted. When compiling the sample of fruit type contrasts, we first used general systematic compilations (Kubitzki 1990–2004, Watson and Dallwitz 1992, Heywood 1993, Mabberley 1997) to find families containing fleshy-fruited species. Apart from the word ‘fleshy’, we also looked for ‘arillate’, ‘baccate’, ‘berry’ and ‘drupe’. As some compilations only give fruit type, some taxa with, for example, capsules with arillate seeds, may have been incorrectly coded as non-fleshy. Since such mistakes have a conservative effect on the analyses, they reduce our chances of finding differences between the contrasted clades. The list of fleshy fruited taxa then passed through two filters: First, we needed updated phylogenies to be able to locate the sister clade to the fleshy fruited clade (references to the phylogenetic hypotheses are found in Table 1), i.e. we only included PhyMPs where we explicitly could locate the phylogenetic node of fruit type change using published phylogenetic hypotheses. In addition to the references to these phylogenetic studies, Kubitzki (1990–2004) and Mabberley (1997) were consulted to be able to add taxa that belonged to the sister clades defined by, but not explicitly included in, the phylogenetic studies. Second, we needed seed mass data, which was sampled from the data compiled by A. T. Moles and the Royal Botanical Gardens Kew’s Seed Information Database (<www.rbgekew.org.uk/data/sid/>). See Moles et al. (2005b) for a detailed description of the seed size data set. The constructed PhyMPs included seed mass data for 3092 species and were spread over the whole range of the angiosperm phylogeny, from magnoliids to euasterids II sensu APGII (2003), representing 24 out of 45 of the APGII orders.

In a few cases there was a secondary fruit type shift within one of the sister clades in a fruit type contrast (PhyMP A). This secondary fruit shift was used to construct another fruit type contrast (PhyMP B). However, since PhyMP B was nested within PhyMP A, all taxa included in PhyMP B were excluded from PhyMP A before the analyses. This is the procedure recommended by Felsenstein (2004, Fig. 25.7, p. 444), and it affected PhyMPs number 27, 42, 53, 54 and 58 (Table 1) in the present study.

In total, 59 PhyMPs were available for the fruit type contrasts (Table 1). For 24 of the PhyMPs, the phylogenetic studies used (Table 1) included reconstructions of fruit type evolution and thereby information on the ancestral state of the fruit type. For another 10 PhyMPs, the fruit type shifts were so deeply imbedded in a clade otherwise characterized by a conserved fruit type that it was considered uncontroversial to deduce the direction of the local fruit type shift (PhyMPs no. 10–13, 18, 29, 31, 37, 45, 47; Table 1). Thus, using this set of 34 PhyMPs we explored whether seed mass variation was dependent on the direction of fruit type shift.

Table 1. An overview of the sample of phylogenetically independent matched pairs (PhyMPs) of fleshy vs. non-fleshy fruited taxa, and derived seed mass values.

PhyMP no.	APG II 'superorder'	APG II order	APG II family	Sampled taxa for each PIMP and its outgroup		Ancestral fruit type	Seed mass (log mg)						PhyMP no.		
				Sample size			Mean		Median		Minimum				
				Nf	F		Nf	F	Nf	F	Nf	F			
1	Magnoliids	Laurales		Atherospermataceae	Siparunaceae	4	1	0.791	0.904	0.942	0.904	0.200	0.905	1	
2	Magnoliids	Laurales		Calycanthaceae	Lauraceae, Monimiaceae, Hernandiaceae	4	108	2.658	2.730	1.974	2.701	1.774	0.161	2	
3	Magnoliids	Magnoliales		Magnoliaceae	Eupomatiaceae, Annonaceae	F	25	2.005	2.331	2.091	2.382	0.889	1.451	3	
4	Magnoliids	Piperales		Sauraceae	Piperaceae	Nf	1	26	-0.453	-0.395	-0.385	-0.452	-1.398	4	
5	Monocots	Dioscorales	Dioscoreaceae	Dioscorea	Tamus	Nf	2	1	0.671	1.248	0.671	1.248	0.314	1.248	5
6	Monocots	Alismatales		Juncaginaceae, Alismataceae, Hydrocharitaceae	Araceae	Nf	14	45	-0.316	0.542	-0.366	0.930	-0.886	-1.215	6
7	Monocots	Liliales	Liliaceae	Calochortus	Streptopus	F	14	1	0.322	0.356	0.297	0.356	0.010	0.357	7
8	Monocots	Liliales	Liliaceae	Erythronium, Lilium, Gagea, Fritillaria	Medeola, Clintonia	F	32	4	0.520	0.687	0.543	0.736	-0.499	0.461	8
9	Monocots	Asparagales		Agavaceae	Ruscaceae	Nf	24	3	1.081	1.871	1.163	2.501	-0.121	0.576	9
10	Eudicots	Ranunculales	Ranunculaceae	Actaea racemosa	Actaea rubra, A. spicata	Nf	1	2	0.447	0.806	0.447	0.806	0.447	0.805	10
11	Core Eudicots	Caryophyllales		Nyctaginaceae	Phytolaccaceae	Nf	28	9	1.146	0.677	1.171	0.784	-0.708	-0.721	11
12	Core Eudicots	Caryophyllales		Portulacaceae, Basellaceae	Cactaceae	Nf	37	25	-0.620	0.667	-0.699	0.686	-2.000	-0.112	12
13	Core Eudicots	Saxifragales		Saxifragaceae	Grossulariaceae	Nf	31	27	-1.210	0.343	-1.307	0.321	-1.903	-0.328	13
14	Rosids	Myrtales	Myrtaceae	Dilleniaceae	Vitaceae	Nf	21	30	0.969	1.454	0.837	1.480	0.160	0.999	14
15	Rosids	Myrtales	Myrtaceae	Backhousia	Plidlostigma, Eugenia, Rhodamnia, Calyptanthus	Nf	2	29	1.009	1.713	1.009	2.075	0.905	-1.201	15
16	Rosids	Myrtales	Onagraceae	Circaea	Fuchsia	Nf	3	2	0.062	-0.946	0.288	-0.946	-0.402	-1.158	16
17	Rosids	Myrtales	Memecylaceae, Melastomataceae	Desmoscelis, Dissotis, Macairea, Melastoma, Pterandra, Pterolepis, Rhexia, Schwackaea, Siphantha, Sonerila, Tibouchina	Memecylon, Mouriri	Nf	17	3	-1.295	1.971	-1.222	1.988	-2.301	1.762	17
18	Eurosids I		Zygophyllaceae	Sisymbrium	Balanites	Nf	1	1	1.606	3.056	1.606	3.056	1.606	3.057	18

Table 1. (Continued)

PhyMP no.	APG II 'superorder'	APG II order	APG II family	Sampled taxa for each PIMP and its outgroup		Ancestral fruit type	Sample size			Seed mass (log mg)						PhyMP no.	
				Nf	F		Nf	F	Nf	F	Mean		Median		Minimum		
											Nf	F	Nf	F	Nf		F
19	Eurosids I	Malpighiales	Malpighiaceae	Thryallis	Bunchosia	Nf	1	3	2.064	2.396	2.064	2.426	2.064	2.321	19		
20	Eurosids I	Rosales	Ulmaceae	Holoptelea	Ampelocera	F	1	2	1.526	2.458	1.526	2.458	1.527	2.294	20		
21	Eurosids I	Rosales	Cannabaceae, Celtidaceae	Cannabis, Humulus	Celtis	F	3	9	0.979	1.800	1.255	1.801	0.421	1.446	21		
22	Eurosids I	Rosales	Rhamnaceae	Urticaceae	Moraceae	F	40	85	-0.142	1.119	-0.256	1.079	-2.426	-1.097	22		
23	Eurosids I	Rosales	Rhamnaceae	Ventilago	Karwinskia, Condalia, Berchemia, Rhamnus	F	2	18	1.360	1.343	1.360	1.229	1.097	0.836	23		
24	Eurosids I	Rosales	Rosaceae	Kageneckia	Amelanchier, Cotoneaster, Mespilus, Pyrus, Pyracantha, Sorbus, Crataegus	F	1	74	0.623	1.351	0.623	1.411	0.623	0.301	24		
25	Eurosids I	Rosales	Rosaceae	Sorbaria, Adenostoma, Spiraea, Holodiscus, Aruncus	Prunus	F	7	53	-0.776	2.418	-1.023	2.317	-1.097	1.442	25		
26	Eurosids I	Rosales	Rosaceae	Rhodotypos, Kerria	Oemleria	F	3	1	1.367	2.277	1.371	2.277	1.045	2.277	26		
27	Eurosids I	Rosales	Rosaceae	Alchemilla, San-guisorba, Chamaerhodos, Acaena, Ivesia, Tetraglochin, Horkelia, Aphanes, Potentilla, Agrimonia	Rosa	F	65	25	-0.355	1.094	-0.495	1.148	-1.620	0.621	27		
28	Eurosids I	Rosales	Rosaceae	Geum, Fallugia	Rubus	F	9	60	0.078	0.152	0.103	0.084	-0.161	-0.523	28		
29	Eurosids II	Brassicales	Brassicaceae	Moringaceae	Caricaceae	F	2	6	2.330	1.538	2.330	1.510	2.305	1.278	29		
30	Eurosids II	Brassicales	Capparidaceae, Brassicaceae, Gyrostemonaceae, Resedaceae	Brassicaceae	Apophyllum, Boscia, Capparis, Crateva, Ritichia	F	278	17	-0.225	1.923	-0.236	2.024	-1.592	0.809	30		
31	Eurosids II	Malvales	Malvaceae	Pavonia, Abelmoschus	Malvaviscus	F	6	1	0.781	1.754	0.666	1.754	-0.638	1.754	31		
32	Eurosids II	Malvales	Thymeleaceae	Thymelaea	Daphne	F	1	2	0.029	2.319	0.029	2.319	0.029	1.927	32		
33	Eurosids II	Sapindales	Rutaceae	Ruta, Chloroxylon	Glycosmis, Aegle, Clausena	F	3	4	0.552	2.074	0.294	2.043	0.114	1.857	33		

34	Asterids	Cornales	Losaceae, Hydrangeaceae Theaceae	18	27	-0.357	1.855	-0.476	1.724	-1.431	0.781	34	
35	Asterids	Ericales	Samolus	9	9	1.979	1.815	2.657	1.463	0.620	-0.018	35	
36	Asterids	Ericales	Theophrasta- ceae, Primulaceae s.str.	1	5	-1.614	2.002	-1.614	2.301	-1.613	1.269	36	
37	Asterids	Ericales	Myrsinaceae	Nf §	16	23	-0.400	1.593	-0.293	1.591	-1.398	0.659	37
38	Asterids	Ericales	Ericaceae	Nf	31	27	-1.242	1.020	-1.155	1.022	-2.500	0.447	38
39	Asterids	Ericales	Ericaceae	Nf	9	1	-1.440	-0.048	-1.454	-0.048	-1.812	-0.048	39
40	Asterids	Ericales	Ericaceae	Nf	6	13	-1.301	0.615	-1.363	0.692	-1.699	-0.229	40
41	Asterids	Ericales	Ericaceae	Nf	3	17	-1.495	-0.552	-1.658	-0.552	-2.046	-1.068	41
42	Euasterids I	Gentianales	Apocynaceae	Nf	2	1	0.470	1.478	0.470	1.478	0.153	1.479	42
43	Euasterids I	Gentianales	Rubiaceae		2	9	-0.147	0.823	-0.147	0.815	-0.323	0.149	43
44	Euasterids I	Gentianales	Rubiaceae		37	2	0.054	0.895	-0.023	0.895	-1.022	0.613	44
45	Euasterids I	Gentianales	Rubiaceae	F §	1	22	-1.770	1.632	-1.770	1.913	-1.770	0.000	45
46	Euasterids I	Lamiales	Gesneriaceae		1	1	-1.820	0.640	-1.820	0.640	-1.819	0.641	46
47	Euasterids I	Lamiales	Lamiaceae	Nf §	2	1	0.346	0.857	0.346	0.857	0.279	0.857	47
48	Euasterids I	Lamiales	Lamiaceae		26	5	0.028	1.785	-0.022	1.641	-0.485	0.226	48
49	Euasterids I	Lamiales	Oleaceae	Nf	1	4	0.627	1.979	0.627	2.033	0.627	1.619	49
50	Euasterids I	Lamiales	Oleaceae	Nf	2	6	0.903	1.325	0.903	1.302	0.766	1.199	50
51	Euasterids I	Lamiales	Oleaceae	Nf	18	12	1.619	2.267	1.581	2.238	1.214	1.777	51

Table 1. (Continued)

PhyMP no.	APG II 'superorder'	APG II order	APG II family	Sampled taxa for each PIMP and its outgroup		Ancestral fruit type	Sample size		Seed mass (log mg)						PhyMP no.
				Nf	F		Nf	F	Mean		Median		Minimum		
									Nf	F	Nf	F	Nf	F	
52	Euasterids I	Solanales	Solanaceae	Hyoscyamus	Atropa	F	3	2	-0.476	0.022	-0.222	0.022	-1.000	<0.001	52
53	Euasterids I	Solanales	Solanaceae	Datura	Physalis, Capsicum, Solanum, Mandragora, Withania	F	8	65	1.009	0.227	1.084	0.176	0.663	-0.377	53
54	Euasterids I	Solanales	Solanaceae	Nicotiana	Lycium, Nicandra	Nf	13	10	-1.096	-0.038	-1.012	-0.075	-1.699	-0.480	54
55	Euasterids II	Aquifoliales	Aquifoliaceae	Cardiopteridaceae	Aquifoliaceae	Nf	1	21	1.041	0.840	1.041	0.966	1.041	-0.620	55
56	Euasterids II	Apiales	Araliaceae	Hydrocotyle, Trachymene	Araliaceae senza Hydrocotyle and Trachymene	Nf	13	29	-0.194	0.712	-0.056	0.778	-1.155	-0.125	56
57	Euasterids II	Apiales	Apiaceae	Centella, Micropleura	Mackliniaya	Nf	1	1	0.217	1.650	0.217	1.650	0.218	1.650	57
58	Euasterids II	Asterales	Rousseaceae	Goodenia, Brunonia, Scaevola	Dampiera	Nf	20	2	0.460	0.571	0.346	0.571	-0.235	0.384	58
59	Euasterids II	Asterales	Argophyllaceae, Alseuosmiaceae	Corokia	Alseuosmia	Nf	1	2	1.785	0.572	1.785	0.572	1.785	0.531	59

Nf means non-fleshy and F fleshy. APG II refers to classifications used in APG II (2003). Nomenclature of the sampled taxa for each PhyMP and outgroup follow the references used to construct the matched pairs. § in the 'Ancestral fruit type' column PhyMPs indicate where we inferred the direction of the fruit type shift from the given phylogenetic hypotheses, while in all others tracing of the ancestral fruit type was part of the cited studies.

The following references were used to construct each matched pair: PhyMP no. 1 (Renner 1999; Sauquet et al. 2003), no. 2 (APGII et al. 2003, S. Renner, pers. comm; Kessler 1993; Renner 1999), no. 3 (Sauquet et al. 2003), no. 4 (Smith 2001), no. 5 (Caddick et al. 2002), no. 6 (Tamura et al. 2004), no. 7 (Patterson and Givnish 2002; Tamura et al. 2004), no. 8 (Patterson and Givnish 2002), no. 9 (Tamura et al. 2004), no. 10 (Compton et al. 1998), no. 11-12 (Cuenoud et al. 2002), no. 13 (Fishbein et al. 2001), no. 14 (Ingrouille et al. 2002), no. 15 (Wilson et al. 2001), no. 16 (Berry et al. 2004; Levin et al. 2003), no. 17 (Clausing et al. 2000; Renner 1989; Renner 1994, S. Renner, pers. comm.), no. 18 (Sheahan and Chase 2000), no. 19 (Davis et al. 2001), no. 20-22 (Syrma et al. 2002), no. 23 (Richardson et al. 2000), no. 24-26 (Porter et al. 2002), no. 27 (Eriksson et al. 2003), no. 28 (Alice and Campbell 1999; Eriksson et al. 2003; Smedmark and Eriksson 2002), no. 29 (Olson 2002), no. 30 (Hall et al. 2002; Rodman et al. 1998), no. 31 (Pfeil et al. 2002), no. 32 (Van der Bank et al. 2002), no. 33 (Chase et al. 1999), no. 34 (Fan and Xiang 2001; Xiang et al. 2002), no. 35 (Luna and Ochoterena 2004; Prince and Parks 2001), no. 36-37 (Källersjö et al. 2000), no. 38 (Kron et al. 2002; Stephens et al. 2004, T. Oliver, pers. comm.), no. 39-41 (Kron et al. 2002), no. 42 (Sennblad and Bremer 2002), no. 43 (Anderson et al. 2001), no. 44 (Andersson and Rova 1999), no. 45 (Rova et al. 2002; Bremer and Eriksson, in ms), no. 46 (Smith 2000), no. 47-48 (Wågstaff et al. 1998), no. 49-51 (Wallander and Albert 2000), no. 52-54 (Knapp 2002), no. 55 (Bremer et al. 2002), no. 56-57 (Chandler and Plunkett 2004; Plunkett et al. 2004), no. 58-59 (Lundberg and Bremer 2003).

Analyses

Seed mass evolution may be manifested either by shifts in the upper or lower boundary of the range, or by changes in average seed mass. Therefore, we tested for differences in minimum, mean, and median of log-transformed seed mass between fleshy and non-fleshy fruited sister clades. We used a paired Wilcoxon signed rank test to determine whether there was a phylogenetically consistent association between fruit type and seed mass. We determined whether the direction of the fruit type shift affected the evolution of seed mass and the associations between fruit type and seed mass by repeating the paired Wilcoxon signed rank test on subsets of the data for which the ancestral fruit type was known (Table 2). We also used t-tests to determine whether a) the direction of fruit type change affected the magnitude of differences in seed mass between sister clades, and b) whether seed mass differed between clades that had the same fruit type but different ancestral fruit state. All tests were performed in R ver. 2.4.0 (R Development Core Team 2006).

Results

There was a consistent association between fleshy fruits and heavier seeds in the present phylogenetically independent matched pairs (PhyMPs) data set (Fig. 1, Table 2). In 49 out of 59 PhyMPs, the fleshy fruited clade had larger median seed mass than its sister clade with non-fleshy fruits. The association between fleshy fruit types and heavier seeds was also corroborated by analyses based on mean, as well as minimum seed masses (Table 2). On average fleshy fruited taxa had 9.5 times higher seed mass compared to their non-fleshy fruited sister taxa, and the differences in mean seed mass between fleshy and non-fleshy fruited clades ranged up to 3.6 orders of magnitude.

The association between fleshy fruit types and heavier seeds was found both when fleshy and non-fleshy fruit types evolved (Fig. 1, Table 2). There was one exception to this trend (Table 2; last row), but this may be an artefact caused by low statistical power ($n = 6$). The consistency of the association between large seeds and fleshy fruit (regardless of the direction of change) shows that seed mass tends to increase in clades that evolve fleshy fruit, and tends to decrease in clades that evolve non-fleshy fruit.

The magnitude of the difference in seed mass between fleshy and non-fleshy fruited clades was not affected by the direction of fruit type change, regardless of seed mass measure (median seed mass: $t = 0.13$, $DF = 13.6$, $p = 0.90$). Seed mass did not differ between clades with the same fruit type but different ancestral fruit types (non-fleshy fruit type; median seed mass: $t = 0.33$, $DF = 17.8$, $p = 0.74$; fleshy fruit type; median seed mass: $t = 0.56$, $DF = 17.9$, $p = 0.58$).

Discussion

In this comparison of fleshy fruited versus non-fleshy fruited plants, a consistent association was found between fleshy fruit

types and higher seed mass. The pattern was supported by >80% of the 59 phylogenetically independent matched pairs included, and the sample ranged from magnoliids to euasterids. This result corroborates previous findings (Hughes et al. 1994, Moles et al. 2005a). Furthermore, the seed mass/fruit type association was not dependent on the direction of fruit type change; seed mass decreased in clades where non-fleshy fruit types evolved and increased in clades where fleshy fruit types evolved. Therefore, it is reasonable to suggest that any general explanation of the evolution of fleshy fruits and endozoochorous seed dispersal in angiosperms must consider seed mass.

Comparative analyses of seed mass have often found differences between plants from different categories based on, for example morphology, habitat, or taxonomy (Salisbury 1942, Harper et al. 1970, Baker 1972, Hodgson and Mackey 1986, Mazer 1989, Hughes et al. 1994, Lord et al. 1995, Jakobsson and Eriksson 2002). However, the large overlaps in seed mass distributions between categories have raised concerns about overly simplistic explanatory models (Hughes et al. 1994, Leishman et al. 2000, Coomes and Grubb 2003). As the present study, by its design, explicitly acknowledged the unique evolutionary context of each phylogenetic lineage, the frequent occurrence of higher seed masses in fleshy fruited lineages makes it relevant to discuss actual causal links between seed mass and fleshy fruit evolution. However, it was beyond the scope of this study to optimize seed mass evolution around each phylogenetically independent fruit shift, and therefore we were not able to disentangle if any of the trait shifts, fruit type or seed mass, preceded the other. Instead, we discuss both alternatives.

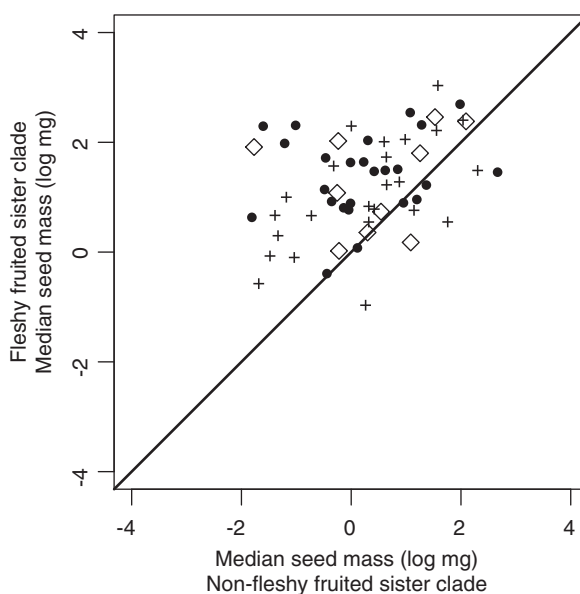


Figure 1. Median seed masses for phylogenetically independent matched pairs of fleshy versus non-fleshy fruited sister clades. + refers to comparisons where fleshy fruit types evolved; ◇ refers to comparisons where non-fleshy fruit types evolved, while direction of fruit type change was not assigned to those represented by ●

Table 2. Paired Wilcoxon signed ranks tests of differences in seed mass between phylogenetically independent matched pairs (PhyMPs) of fleshy versus non-fleshy fruited sister clades. Upper values concern the whole data set and lower values concern those comparisons where both sister clades were represented by three or more species.

Ancestral fruit type	Sample size	Mean seed mass	Median seed mass	Minimum seed mass
Mixed	59	***	***	***
	28	***	***	***
Non-fleshy	24	**	**	**
	9	**	**	**
Fleshy	10	*	*	*
	6	0.16	0.22	0.22

Significant differences are denoted as *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Why should seed mass increase after evolution of fleshy pulp?

Seeds dispersed by endozoochory need to survive a mouth and gut passage. The increase in seed mass may thus reflect an improved defense of the seed against the mechanical and chemical wearing during this passage, either due to a reduced surface/volume ratio, a thicker seed coat, or evolution of a seed enclosed in an endocarp (in drupes). When the relation between increased defensive seed tissue and seed predation was examined, though without considering dispersal mode, Moles et al. (2003) found no effect of seed mass or of the proportion of defensive seed tissue on pre- and post-dispersal seed predation. However, frugivory and gut passage of seeds add some complexity to the issue of defensive tissues. Gut passage has been found to increase germination success, but only in fleshy fruited species. Seeds sampled from dry fruits generally showed decreased germination after gut passage (Traveset and Verdu 2002, Cosyns et al. 2005). Increasing gut retention time has a negative effect on the proportion of germinating seeds both among fleshy fruited and non-fleshy fruited taxa (Murray et al. 1994, Charalambidou et al. 2003), and Levey and Grajal (1991) showed that seed size is negatively correlated with gut retention time. Taken together, these results suggest that survival through gut passage may have selected both for a more protective seed coat and increased seed size, to diminish detrimental abrasion during gut passage. They also suggest that endozoochory should favor an improved protective ability in smaller seeds as their gut passage is longer. As far as we know, this has never been tested for endozoochorous taxa specifically, but in Moles et al. (2003), where all dispersal modes were lumped, the slope of the relationship between seed reserve mass and defensive tissue was 1.07, i.e. the relative mass of defensive tissue was no greater in smaller seeds.

Most present-day fleshy fruited plants rely on birds and mammals for seed dispersal. Due to their foraging and feeding behavior seeds are generally dispersed to understory sites or at least below shrubs or trees (Debussche and Isenmann 1994, Jordano and Schupp 2000) where frugivores retreat while digesting fruit. Evolving the fleshy pulp may thereby secondarily lead to a selection pressure for higher seed mass, as heavier seeds seem to be favored in understory recruitment conditions (Leishman et al. 2000). Tiffney (1984, 2004) reasoned along this line when developing his hypoth-

esis for the dramatic increase in seed sizes and fleshy fruits in the Early Tertiary. He argued that plants exploiting the radiating mammalian and avian fauna got the opportunity to evolve heavier seeds and hence colonize and develop a more closed vegetation.

Why should a fleshy pulp evolve after an increase in seed mass?

The intuitive answer to this question is that fleshy fruits evolved to ensure adequate dispersal distance for heavier falling seeds by taking advantage of frugivore movements. Two different mechanisms may explain why heavier seeds lead to a decrease in dispersal ability. First, it is likely that heavier seeds travel shorter distances unless they are aided by a biotic seed dispersal agent. Second, an evolutionary shift to heavier seeds comes with the cost of reduced seed number per unit energy invested, due to the resource-limitation based seed mass/seed number tradeoff (Harper et al. 1970, Smith and Fretwell 1974, Geritz 1995, Rees and Westoby 1997, Coomes and Grubb 2003, Moles and Westoby 2006). Producing a lower number of seeds implies a decrease in dispersal ability as fewer potential recruitment sites are reached. However, an interspecific comparison showed that the expected loss of seed number does not accompany increases in seed mass among angiosperms in general, because seed mass evolution is strongly correlated with shifts in plant size (Moles et al. 2004, 2005a, Moles and Westoby 2006). We are therefore more inclined to suggest the decrease in dispersal-by-distance than a decrease in dispersal-by-numbers as the potential driver behind the association between evolution of fleshy fruit types and heavier seeds.

In the seed dispersal literature, three different dispersal strategies have been proposed: 'colonization', 'directed', and 'escape' dispersal (reviewed by Howe and Smallwood 1982). The 'colonization' strategy has been considered most relevant for plants with numerous small seeds, typically found early in successional sequences and in ruderal habitats. Obviously this strategy does not fit with the shift to heavier seeds found here for fleshy fruited plants. The second dispersal strategy, 'directed' dispersal, has been of particular interest in relation to fleshy fruited plants, as the non-random movements of frugivores opens the opportunity for dispersal directed to a certain category of sites. The destinations that have mainly

been considered reasonable for frugivore-directed seed dispersal are forest margins and tree-fall gaps (Hoppes 1988, Levey 1988, Wenny and Levey 1998, Jordano and Schupp 2000). We are not aware of any literature that suggests why the conditions of these forest margin habitats should motivate a shift to heavier seeds. Thus, an association between the evolution of fleshy fruits and the 'directed' dispersal strategy does not seem generally plausible. In desert habitats, though, directed dispersal towards crowded and thus shadier islands of vegetation could have favored the simultaneous evolution of larger seeds and fleshy fruit types in some plant lineages. The third dispersal strategy, 'escape', refers to Janzen's (1970) and Connell's (1971) idea of dispersal away from the mother plant. This strategy does not invoke any other qualitative aspect of dispersal than to avoid increased risk of mortality in the vicinity of the mother plant (Harms et al. 2000). As heavier seeds will fall closer to the mother plant, plants evolving heavier seeds may get involved in such distance-dependent selection exerted by sibling competition or, for example, parasites, herbivores, predators etc. hosted by the mother plant. If so, selection will favor an evolutionary shift in resource allocation towards dispersal attributes parallel to the selection for heavier seeds (Eriksson and Jakobsson 1999). The phylogenetic association between higher seed mass and fleshy fruit types found here thus fits with this 'escape' dispersal strategy.

Frugivore selection

The lack of between-year consistency in plant–frugivore interactions (Herrera 1998), the apparently low frequency of specialized plant–frugivore interactions (Herrera 1982, Wheelwright and Orians 1982), and the allometric plant–fruit size relations (Herrera 2002b) have supported the view that frugivores exert weak selection pressures on fleshy fruit evolution. However, quantitative knowledge of seed disperser effectiveness is scarce (Jordano and Schupp 2000, Wenny 2000b) and there is a strong call for an integrated approach to understand the recruitment effects of frugivore fruit selection and seed dispersal (Herrera et al. 1994, Schupp and Fuentes 1995, Wang and Smith 2002). Even though the present study did not aim to analyze evolution within fleshy fruited taxa, the frequent occurrence of higher seed mass in fleshy fruited clades gives us reason to discuss frugivore selection in relation to seed mass and recruitment ability in fleshy fruited plants. Seed mass is a key trait for recruitment, and higher seed mass is often positively correlated with seedling survival (Leishman et al. 2000), particularly in understory habitats characterized by shade, litter and falling debris, i.e. habitats where we typically find present-day fleshy fruited species (Willson et al. 1990, Guitián and Sanchez 1992, Herrera 2002a, Patterson and Givnish 2002, Bolmgren and Eriksson 2005). Fleshy fruited plants growing in more closed vegetation types may thus be exposed to a prevailing selection pressure for heavier seeds. However, as the association between heavier seeds and fleshy fruits suggests, dispersal ability is likely to be an important concern for plants with heavier seed mass. If this is true, seed mass evolution in fleshy fruited plants may be constrained by the availability of larger frugivores that can han-

dle heavier and larger seeds and fruits (Wheelwright 1985). Fruit size is an important factor in removal success (Sallabanks 1993, Jordano 1995a, Rey et al. 1997, Alcántara and Rey 2003) and the fruit size–frugivore size correlation is the most consistent correlation among plant–frugivore interaction traits (Jordano 1995b, 2000, Herrera 2002a). It is reasonable to believe that the strength of frugivore selection, and particularly gape-limited seed mass evolution, will be positively correlated with the closedness of the surrounding vegetation. In this way, present-day dynamics in fleshy fruit evolution may mirror the historical dynamics of fleshy fruit evolution, particularly the conspicuous increase in seed mass and proportion of fleshy fruits in times when tropical-like forests expanded in the Early Tertiary (Wing and Tiffney 1987, Tiffney 1984, 2004, Eriksson et al. 2000, Eriksson 2008).

Conclusion

Using an angiosperm-wide sample of fleshy versus non-fleshy fruited sister clades, this study found a consistent association between fleshy fruit types and higher seed mass. The direction of fruit type change did not affect this pattern, as seed mass was higher in clades where fleshy fruits evolve and lower in clades where non-fleshy fruits evolved. Among the original hypotheses on the evolution of fleshy fruits (Snow 1971, McKey 1975, Howe and Estabrook 1977, Howe and Smallwood 1982, Cipollini and Levey 1997), only dispersal related hypotheses have considered seed mass. Our results fit with the idea that fleshy fruits evolved to maintain an 'escape' type of dispersal (*sensu* Howe and Smallwood 1982), counteracting the negative effects of heavier seeds traveling shorter distances. Increased seed mass may also represent an allocation to protective tissues to survive ingestion. More specific hypotheses, related to the Early Tertiary increase in seed mass and fleshy fruit types, consider seed mass increase as a cause (Eriksson et al. 2000) or an effect (Tiffney 1984, 2004) of fleshy fruit evolution. Moles et al. (2005a) suggested that seed mass increase was an effect of life form evolution associated with long life span, with no direct coupling to fruit type. As it was beyond the scope of this study to disentangle the order of fruit type and seed mass shifts, we cannot discriminate between these hypotheses. However, evolutionary divergences in seed mass have been more strongly correlated with growth form than with changes in fruit type (Moles et al. 2005a). This suggests that changes in fruit type are not the main driver of change in seed mass, and provides some support for the idea that changes in seed mass might generally precede changes in fruit type. The consistent association between seed mass and fleshy fruits calls for studies of seed disperser effectiveness where gape width, fruit size, and seed mass are examined in relation to seed fall patterns and recruitment success.

Acknowledgements – This study was supported by grants from the Swedish Research Council. Seed mass data was kindly provided by Angela Moles and John Dickie, and was used with the permission of the Trustees of the Royal Botanic Gardens, Kew. The Millennium Seed Bank Project is funded by the UK Millennium Commission, The Wellcome Trust and Orange PLC; The Royal Botanic Gardens Kew are partially funded by the UK Dept of Environment, Food and Rural Affairs.

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