Evolutionary and ecological correlates of early seedling morphology in East African trees and shrubs[1]

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Seed size and cotyledon morphology are two key juvenile traits that have evolved in response to changes in plant species life-history strategies and habitat associations. Correlations of these traits with each other and with other juvenile traits were examined for 70 species of trees and shrubs in Kibale National Park, Uganda. Although species with photosynthetic cotyledons were more abundant than in other tropical floras, both univariate and multivariate analyses supported trait associations expected from the literature. Trait values varied continuously across species, yet mean trait values differed significantly among habitat association types. Species with large seeds, large seedlings, thick storage cotyledons, slow germination, large-stature adults, and dispersal by large animals were common in forest and gap habitats. An opposite suite of traits was common in open habitats (grassland and edge). Analyses incorporating phylogeny (independent contrasts and omnibus tests) confirmed that these suites of traits showed correlated evolution. Cotyledon functional morphology yielded a strong phylogenetic signal, while seed mass was labile. Nevertheless, contingent change tests found that evolutionary change from photosynthetic to reserve cotyledons was more likely when disperser and perhaps seed size of ancestral species were already large, suggesting a strong interdependency among these traits.

Key words: cotyledon morphology; habitat; Kibale National Park; life history; phylogeny; seed size; Uganda.

Plant species differ greatly in seed and seedling traits, and these traits are often associated with regeneration in particular habitats (Kitajima and Fenner, 2000; Leishman et al., 2000). For example, specialization for gap regeneration appears to select for small seeds that are more likely to be dispersed far from the parent tree (Foster, 1986; Dalling et al., 1998), while large seeds are more likely to be dispersed by large vertebrates to shaded habitats where they have to depend for prolonged periods on energy and nutrients in reserve cotyledons (Osunkoya, 1996; Hewitt, 1998; Kitajima and Fenner, 2000; Leishman et al., 2000; Westoby et al., 2002). While seed size determines the amount of resources initially available to a seedling, cotyledon functional morphology determines how these resources are used during initial seedling growth and development (Hladik and Miquel, 1990; Garwood, 1996; Kitajima, 1996; Green and Juniper, 2004). Species with photosynthetic cotyledons start using light as an energy source earlier than those with reserve cotyledons (Kitajima, 2002). Thus, photosynthetic cotyledons are more advantageous in high-light environments where small seed size can be easily compensated

for by faster autotrophic growth (Garwood, 1996). Reserve cotyledons, on the other hand, provide resources to support seedling energy demands during times of stress and may be an adaptation to growing in low light (Ibarra-Manríquez et al., 2001). In general, a strong correlation exists between seed size and cotyledon functional morphology across species, such that the inverse of cotyledon thickness, which indicates the degree of photosynthetic differentiation, is negatively correlated with seed size (Kitajima, 1996).

Nevertheless, the current selective regime is not the only determinant of seed size and cotyledon morphology relationships; historical or phylogenetic relationships may also strongly influence individual traits and their correlations (Silvertown and Dodd, 1996; Ackerly and Reich, 1999), such that closely related species show strong resemblances. Cotyledon morphology has been suggested to be more phylogenetically conserved than seed size (Garwood, 1996; Kitajima and Fenner, 2000). If cotyledon morphology indeed shows a stronger phylogenetic signal than seed size, phylogenetically uncorrected relationships between habitat and cotyledon morphology are predicted to be weaker than relationships between habitat and seed size. To date, though, phylogenetic influences on these juvenile traits have been poorly studied. Now, with the availability of stronger hypotheses of angiosperm phylogenies (Soltis et al., 2000) and analyses to investigate changes occurring across phylogenies (Felsenstein, 1985; Garland et al., 1993; Pagel, 1994; Blomberg et al., 2003), we can more critically examine phylogenetic signals in trait relationships.

Here, we report the first study of juvenile functional traits and their associations with habitat for 70 tree and shrub species in Kibale National Park, Uganda. In particular, we examined correlations between seed mass and cotyledon morphology, as well as multivariate relations among these and other functional traits of juveniles (i.e., germination speed, dispersal agent, initial seedling size), adult stature (i.e., growth form, maximum

TABLE 1.   Percentage representation of cotyledon types in Kibale National Park, Uganda (this study) compared to other tropical floras from published studies (means per geographic region followed by SD). Data for the neotropics (N = 5 sites), paleotropic Asia (N = 2 sites), and paleotropic West Africa (N = 2 sites) are summarized from Garwood (1996) and Ibarra-Manríquez et al. (2001). Data for Kibale are from this study and are reported as only forest-associated species (N = 25 species) and species from all habitats (N = 70 species).

Cotyledon type	PEF	PER	CHR	PHR	CER
Neotropics[a]	49.5 (4.8)	15.8 (3.9)	24.1 (6.6)	8.3 (1.0)	2.3 (0.8)
Paleotropics					
Asia[b]	33.8 (1.5)	23.9 (7.3)	23.7 (7.1)	11.0 (1.4)	7.8 (0.3)
West Africa[c]	36.8 (3.2)	34.1 (13.2)	16.7 (7.4)	8.1 (1.3)	4.4 (1.2)
Kibale forest[d]	72.0	4.0	24.0	0.0	0.0
Kibale all habitats[d]	74.3	8.6	15.7	1.4	0.0

Note: PEF = phanerocotylar epigeal foliaceous with cotyledon thickness <0.9 mm, PER = phanerocotylar epigeal reserve with cotyledon thickness >0.9 mm, CHR = cryptocotylar hypogeal reserve, PHR = phanerocotylar hypogeal reserve, CER = cryptocotylar epigeal reserve.

[a] Mexico, Guadeloupe, Panama, Puerto Rico, Mexico.
[b] Indonesia, Malaysia.
[c] Gabon, Nigeria.
[d] Kibale.

adult height), and habitat associations. Using a phylogenetic approach, we assessed the importance of shared evolutionary history in shaping seed and seedling traits. In addition to forests and gaps, open habitats (grassland and edge) were included because they historically and presently comprise a large part of the African landscape (Morley, 2000). Woody species growing in these habitats may have very different traits than species growing in forested habitats. Based on other studies, we predicted that species with large seeds, large seedlings, animal dispersal, and thick storage cotyledons would be most common among large trees growing in closed-canopy forest.

MATERIALS AND METHODS

Study site—Kibale National Park (795 km²; 0°13′–0°41′N and 30°19′–30°32′E) is located in western Uganda, 24 km east of the Rwenzori Mountains at an elevation of approximately 1500 m. Between 1998 and 2000, a mean of 1760 mm of rain fell per year; annual mean daily maximum temperature was 23.1°C, and minimum temperature was 15.1°C. Kibale consists of mature forest (57%), colonizing forest (19%), grassland (15%), woodland (4%), swamp (4%), and plantations of exotic trees (1.0%) (Chapman and Lambert, 2000). The forest is classified as a medium-altitude, moist, tropical forest, transitional between lowland rain and montane with a canopy height averaging 25–30 m (Howard, 1991). Natural disturbances leading to treefall gaps are common (Skorupa and Kasenene, 1984). The forest lacks aggressive colonizers (e.g., *Musanga* spp., *Cecropia* spp.) typical of other tropical regions (Chapman et al., 1999), but other early successional species are present (e.g., *Albizia grandibracteata*, *Polyscias fulva*, *Trema orientalis*; Zanne and Chapman, in press). Grasslands are thought to be anthropogenic in origin, but have been abandoned for at least 50–100 years (Kingston, 1967). Forest has re-established in some grasslands, while the majority are still dominated by grasses, which are maintained by fire, elephant damage, and/or competitive dominance of grasses over trees (Kingston, 1967). To classify species by their habitat association (habitat in which they are typically found as juveniles or adults), we identified three habitat types: closed-canopy forest, treefall gap, and open (grassland and grassland/forest edge).

Species—We included all species for which seeds were available during a 2-yr period (1998–2000; Appendix, see Supplemental Data with online version of this article), encompassing a wide range of seed sizes, cotyledon types, and habitat associations. Species with fruiting frequencies greater than 2 years or at lower densities were more likely to have been missed. We were unable to sample many shrubs (N = 3) and grassland species (N = 3), but patterns found in other growth form and habitat classes were similar to trends found within these groups. Thus we believe the patterns we found are robust. Species names and maximum adult heights were derived from the literature including

Eggling and Dale (1952), Polhill (1952), Hamilton (1991), Katende et al. (1995), and Lwanga (1996). Determination of habitat associations (forest, gap, and open) was primarily based on analyses by Zanne and Chapman (in press), which surveyed species stem densities in 96 plots (5 × 5 m for stems <0.5 m, 0.5–2 m, and >2 m) across the habitat types in Kibale, and assessed the significance of observed habitat associations with a 95% confidence interval calculated by randomization tests (i.e., 10 000 iterations of random reassignment of plots to habitat types). Most habitat associations were the same for the three size classes. Although some species were positively associated with more than one habitat in Zanne and Chapman (in press), each species was assigned to the single habitat category in which it was most frequent and supported by the literature. Growth form (shrubs, treelets, trees) was from Polhill (1952). As few shrubs (N = 3) occurred among the species considered in this study, shrubs and treelets are considered together.

Method—From May 1999 to May 2000, fruits and seeds were collected; seeds from multiple adults were selected whenever possible. A subsample of seeds (average = 37.4, range = 2–650) was separated from fruit pulp and any dispersal appendages (e.g., wings or hairs as in Westoby, 1998), sun dried, and transported to the University of Florida. Seeds were oven dried at 60°C to a constant mass to determine the following traits (Appendix, see Supplemental Data with online version of this article): total seed mass (TSM, including seed coat and part of fruit that remains after removal of dispersal appendages), seed reserve mass (SRM, including embryo plus endosperm), percentage of total seed mass comprised of seed reserve mass (%SRM). Seed dispersal syndrome (small animal, large animal, nonanimal including wind and autochorous) was assessed by seed dimensions, presence of a fleshy fruit or aril, and feeding observations of large and small animals (Zanne, 1998). Seed collection, storage, and germination guidelines followed Katende et al. (1995).

A second subsample of seeds for each species was germinated in eight raised nursery beds (1 × 13 m) in Kibale. Three degrees of shading were made using differing amounts of dried thatch, with six of the beds experiencing heavy shade and one bed each experiencing medium and light shade. Photosynthetically active radiation (PAR) values at 1200 h in June 2000 were determined, with eight locations measured on each bed and two readings taken per location. Light averaged 27 μmol · m⁻² · s⁻¹ (heavy shade), 182 μmol · m⁻² · s⁻¹ (medium shade), and 367 μmol · m⁻² · s⁻¹ (light shade). To facilitate germination, seeds were planted under shading that matched their habitat association. The number of days until germination and percentage germination (measured when seeds were all germinated or obviously rotten) were recorded. During the dry season, seedbeds were watered every 2–3 days by saturating the surface soil (~2.1 L/m²).

Cotyledon type was classified as in Garwood (1996) and described by a three-letter code (Table 1). The first letter of the code refers to cotyledon exposure: C (cryptocotylar = inside seed coat) vs. P (phanerocotylar = free

of seed coat). The second letter refers to cotyledon position: E (epigeal = aboveground) vs. H (hypogeal = at or belowground). The third letter refers to functional morphology: F (foliaceous = thin and photosynthetic) vs. R (reserve = storage). In this study, we found only PEF, PER, CHR, and PHR. The type CER found in other tropical floras was missing at Kibale. Because PHR (one sp.) was rare, it was grouped together with CHR (11 spp.) as *HR. Seedlings were harvested for determination of initial seedling mass (ISM) when the first photosynthetic organ (either cotyledons or true leaves) became fully expanded. Seedlings were dried at 60°C to constant mass and weighed. Cotyledon thickness was measured at harvest with calipers (Digimatic Caliper, Forestry Suppliers, Inc., Jackson, Mississippi, USA). From this measure, we calculated cotyledon photosynthetic index (CPI) as the inverse of cotyledon thickness for phanerocotylar species, which should be linearly correlated with photosynthetic capacity per cotyledon mass due to a fundamental constraint on light extinction through green photosynthetic tissue (determined for Panamanian seedlings; Kitajima, 1992). To all cryptocotylar species with completely nonphotosynthetic cotyledons, a value of 0/mm was assigned arbitrarily.

Analyses—Sample size varied among tests because we were unable to measure all seed and seedling traits for all species (e.g., seeds were collected but no seedlings germinated). Total seed mass (mean = 1.02 ± 4.06 g) was analyzed as both a continuous and discrete trait; for discrete traits, seed size was divided into four classes based on 10-fold differences in seed mass as in Hughes et al. (1994). To increase normality of distributions of continuous variables, all percentage data were arcsine square-root transformed, and all mass and height data were log_{10}-transformed. Days to germination and cotyledon photosynthetic index were normally distributed without transformation. Seed reserve mass and total seed mass were highly correlated ($r = 0.95$, $P < 0.001$), so only total seed mass was considered in analyses.

We examined associations between cotyledon type and other discrete traits without considering phylogeny with contingency table goodness-of-fit (GOF) tests using programs written in R 1.6.2 (R Development Core Team, 2002) in which P values were computed using Monte Carlo simulations with 10 000 iterations. Only comparisons in which differences were significant in an overall comparison were analyzed further with pairwise GOF tests with appropriate Bonferroni corrections such that, experimentwise, $P = 0.05$. Multivariate trait associations were examined with principal components analyses (PCAs) using Canoco 4.5 (ter Braak and Smilauer, 1999). Because we did not have data for all variables for all 70 species, a compromise between species number and variable inclusion was made. An analysis containing eight variables and 48 species was the best compromise, allowing the most species and variables. Also, because data from regional flora (e.g., growth form, adult height, and some habitat association values) may not reflect values for Kibale, PCAs were run with and without these variables. Results did not differ for the remaining variables for the first axis, and a negative relationship between %SRM and cotyledon photosynthetic index was found along the second axis (data not shown).

A phylogeny of the study species was constructed from the literature (for details see Zanne, 2003). When insufficient data were available to define phylogenetic relationships, relationships were left unresolved. Because branch lengths were unknown, a punctuated model of evolution was assumed that set all branch lengths to 1 except those differentiating polytomies, which were set to 0.0000001. Continuous traits were analyzed with independent contrasts (Felsenstein, 1985) using Compare 4.6 (Martins, 2004). The resulting contrast values were analyzed in the same way as phylogenetically uncorrected values using Pearson product moment correlations and PCA, except that relationships were centered on the origin (Garland et al., 1992; Ackerly and Donoghue, 1998). Because contrast value signs are arbitrary except when relative to one another for traits at a given node, in Fig. 2B using contrast values, all X-value contrasts are made positive, and Y-value contrasts are made positive or negative depending on whether they co-varied positively or negatively with the X-values (Ackerly and Reich, 1999). PDAP 6.0 (Garland et al., 1993; Blomberg et al., 2003) was then used to determine if seed size and cotyledon thickness showed phylogenetic signals (i.e., whether more closely related species showed greater resemblance). The test for phylogenetic signal is based
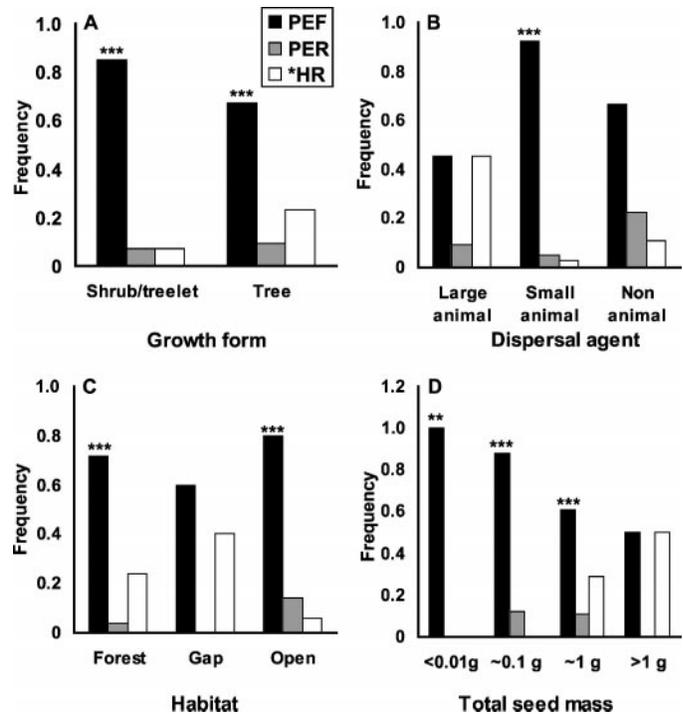

Fig. 1. Frequency of cotyledon types in Kibale National Park, Uganda in relation to four factors. (A) Growth form (shrub/treelet: $N = 27$, tree: $N = 43$). (B) Dispersal agent (large animal: $N = 22$, small animal: $N = 39$, non-animal: $N = 9$). (C) Habitat type (forest: $N = 25$, gap: $N = 10$, open: $N = 35$). (D) Total seed mass (<0.01 g, $N = 6$; ~0.1 g, $N = 25$; ~1 g, $N = 28$; >1 g, $N = 6$). Cotyledon types: PEF = phanerocotylar epigeal foliaceous, PER = phanerocotylar epigeal reserve, *HR = hypogeal reserve. Goodness of fit tests examined significance of bias in prevalence of PEF cotyledons (*$P \leq 0.05$, **$P \leq 0.005$, ***$P \leq 0.0005$).

upon a comparison of the fit of the observed phylogeny (using the variance in the independent contrast values) with a 95% confidence interval of fit values calculated by randomization tests (i.e., confidence interval fit values are the variance in the independent contrast values for 1000 iterations of random reassignment of tip values). Our test values should be less than the critical values if phylogenetic signal occurs in the data. Discrete traits were examined for correlated change using the omnibus test in Discrete 4.0 (Pagel, 1994). Significance was determined using randomizations tests with 1000 iterations. Tests were considered significant if their likelihood ratio between independent and dependent models of trait evolution was greater than 95% of the iterations. Significant correlations were analyzed further with the contingent change test (Pagel, 1994). These tests were considered significant if their likelihood ratio was significant at $P \leq 0.05$ assuming a χ^2 distribution with 1 degree of freedom.

RESULTS

Relationships among discrete traits—Among the study species in Kibale, photosynthetic cotyledons (PEF) were the most frequent, representing a much greater proportion of the flora than in other tropical forests (Table 1). The GOF tests support significant associations between cotyledon types and seed size classes, as well as with other discrete traits (Fig. 1). The PEF cotyledons represented a significantly greater proportion than the PER and *HR cotyledons in both growth form categories, for small-animal dispersal, forest and open habitat associations, and the three smaller seed size categories. The *HR cotyledons (belowground storage) were best represented

(>20% of species) among trees (than among shrubs), large-animal dispersal (than in small-animal or nonanimal dispersal), forest and gap associations (than in open habitat association), and the two larger seed size categories (than in smaller seeds).

Seed size and cotyledon type showed correlated evolutionary changes with one another and with dispersal agent and habitat type, corroborating the ecological associations indicated by the GOF tests. Trait state shifts to large dispersers (omnibus test: $P < 0.00098$), large seeds (omnibus test: $P < 0.00090$), and gap habitats (omnibus test: $P < 0.032$) coincided with trait state shifts to *HR cotyledons. Trait state shifts to large seeds coincided with trait state shifts to forest habitats (omnibus test: $P < 0.050$). Furthermore, contingent change tests suggest that the transition from photosynthetic (PEF) to storage cotyledons (**R) is more likely when the ancestors already have large-animal dispersers ($\chi^2 = 4.66$, $P < 0.05$, df $= 1$) and perhaps very large seeds ($\chi^2 = 3.42$, $P < 0.10$, df $= 1$).

Relationships among continuous traits—Cotyledon thickness was a conserved trait across the phylogeny with closely related taxa showing considerable similarity (phylogenetic signal test: critical value $= 2.74$, test statistic $= 2.19$, $P < 0.001$), but total seed mass did not show a strong signal as it varied considerably across the phylogeny (phylogenetic signal test: critical value $= 0.35$, test statistic $= 0.43$, $P > 0.350$). Indeed, most families had only one cotyledon type, whereas seed size varied in most families by at least two orders of magnitude. Nevertheless, whether or not phylogenetic relationships were incorporated, cotyledon photosynthetic index (CPI, calculated as the inverse of cotyledon thickness) and seed mass were significantly negatively correlated (Fig. 2A raw values, Fig. 2B independent contrast values). Both with and without phylogenetic corrections, seed mass was also significantly positively associated with seed reserve mass, days to germination, initial seedling mass (with and without cotyledons), and maximum adult height, but not with percentage germination and %SRM (Table 2). In all cases, when phylogeny was taken into account, the strength of the correlations between the contrasts was the same or more typically stronger than without phylogeny, with cotyledon thickness showing the greatest increase (Fig. 2).

Multivariate analyses—In the PCA of seed, seedling, and adult traits without incorporating phylogeny, six traits had strong factor loadings on axis 1, with total seed mass contributing the most (Table 3, Fig. 3). Large-seeded species loaded positively on axis 1; they tended to be dispersed by large animals and more frequent in forest and gaps, require more days to germinate, have thick less-photosynthetic cotyledons, and achieve taller adult stature. While habitat associations of the species were related with other traits along axis 1, forest- and gap-associated species (indicated by different symbols in Fig. 3) overlapped considerably with each other in multivariate space. Species were continuously distributed along axis 1, suggesting that this suite of traits varies continuously among species. Eighteen species (38%) were related positively, and 17 species (36%) were related negatively to axis 1. Growth form, which did not load strongly on axis 1, loaded most strongly on axis 2; days to germination and adult height also loaded strongly on axis 2 (Table 3). When the analysis was run with initial seedling mass, which was measured for a subset of 39
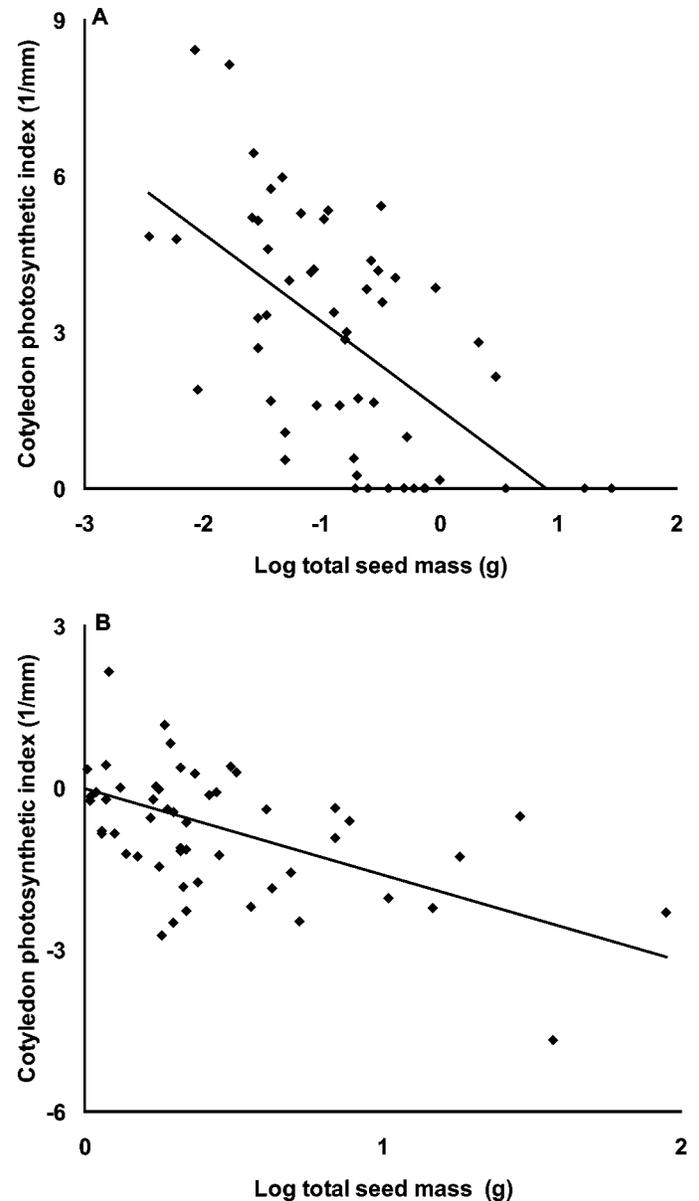

Fig. 2.   Relationship between total seed mass ($\log_{10}$-transformed) and cotyledon photosynthetic index (= inverse of cotyledon thickness in mm) for 53 species in Kibale National Park, Uganda. (A) Without incorporating phylogenetic relationships ($r = -0.59$, $P < 0.001$). (B) Incorporating phylogenetic relationships using independent contrasts ($r = -0.70$, $P < 0.001$).

species, initial seedling mass was also strongly positively related to axis 1.

The PCA incorporating phylogeny (using independent contrast values for continuous traits) also demonstrated the importance of seed mass. Overall, it showed the same multiple trait associations as found in the analysis without incorporating phylogeny, except CPI contributed much more in defining axis 1 (Table 3). Indeed, the absolute value of factor loading of CPI (0.86) was almost as strong as that for total seed mass (0.88). The increase in the strength of CPI was due in part to the removal of the discrete traits, as CPI had an absolute value of factor loading of 0.76 in an analysis with neither discrete traits nor phylogeny incorporated. But CPI with phylogeny

TABLE 2. Pearson product moment correlations between total seed mass and other continuous traits for data when phylogenetic relationships are not incorporated and incorporated using independent contrasts. Significant relationships are in bold.

	Total seed mass			
	Phylogeny not incorporated		Phylogeny incorporated	
Traits	r	P	r	P
Seed reserve mass	**0.95**	**<0.001**	**0.95**	**<0.001**
Germination (%)	0.08	0.639	0.23	0.177
Seed reserve mass (%)	0.01	0.941	−0.02	0.861
Days to germination	**0.39**	**0.006**	**0.40**	**0.004**
Initial seedling mass (with cotyledons)	**0.92**	**<0.001**	**0.94**	**<0.001**
Initial seedling mass (without cotyledons)	**0.89**	**<0.001**	**0.90**	**<0.001**
Cotyledon photosynthetic index (all spp.)	**−0.59**	**<0.001**	**−0.70**	**<0.001**
Cotyledon photosynthetic index (without spp. with cryptocotylar cotyledons)	**−0.42**	**0.005**	**−0.57**	**<0.001**
Maximum adult height	**0.39**	**0.002**	**0.46**	**<0.001**

(0.86) was still stronger than without phylogeny (0.76) incorporated for only the continuous traits, suggesting a much stronger association of seed mass with cotyledon function when phylogeny is incorporated in the analysis. As in the PCA including initial seedling mass without incorporating phylogeny, initial seedling mass was also strongly positively related to axis 1 in the PCA incorporating phylogeny. Adult height and days to germination once again significantly loaded on axis 2.

DISCUSSION

This study demonstrates that seed size and cotyledon morphology, traits indicating the amount of resources available and how resources are utilized by seedlings, respectively, show strong associations with one another, as well as with other juvenile traits and forested vs. open habitats. Earlier studies in other tropical forest communities also found significant associations of seed mass with cotyledon function (Hladik and Miquel, 1990; Garwood, 1996; Kitajima, 1996; Ibarra-Manríquez et al., 2001), but no one has examined the extent to which this association is due to correlated evolutionary change. In our analyses taking phylogeny into consideration, cotyledon functional morphology and seed mass showed even stronger associations with each other than when phylogeny was not incorporated (Fig. 2B, using independent contrast tests). In addition, contingent change tests suggest that evolutionary change in cotyledon morphology is dependent on disperser size and potentially on seed size. Suites of traits associated with both forested (forest and gap) and open habitats (edge

and grassland) were found to be robust after taking phylogeny into account, suggesting contrasting selective pressures between these habitats over evolutionary time.

Seed size and cotyledon type—Differences in total seed mass among species were driven by differences in embryo and endosperm mass (i.e., seed reserve mass) and not differences in seed coat mass (Leishman et al., 2000), suggesting that embryo and reserve size are especially important in seed size relations. Large seeds were most strongly associated with tall trees (Moles et al., 2004) in closed-canopy forest where light availability is low. These results support the widely accepted generalization that size of the large seed reserve supports seedling survival in shade (Garwood, 1996; Leishman et al., 2000; Westoby et al., 2002), although they are in contrast to studies finding negative relationships between shade tolerance and tree stature (Thomas, 1996; Davies et al., 1998; Thomas and Bazzaz, 1999). Surprisingly, however, storage cotyledons (*HR) were less strongly associated with forest than with gaps in our study, suggesting that continued energy dependency on storage cotyledons is not the main mechanism for survival in shade. Indeed, many species with photosynthetic cotyledons can establish and survive for multiple years in the shaded forest understory (A. Zanne, unpublished data for Kibale species; K. Kitajima, unpublished data for Panamanian species). Carbohydrate reserve size in seedling stems and roots is actually a stronger predictor of survival in shade than is cotyledon reserve size (J. Myers and K. Kitajima, unpublished data). Thus, large initial seedling size, rather than prolonged support by

TABLE 3. Eigenvalues and factor loadings for seed, seedling, and adult traits for the first two axes using principal components analyses run without and with phylogeny incorporated for 48 species in Kibale National Park, Uganda. Numbers in bold denote strong factor loadings (>0.50). Cotyledon photosynthetic index includes species with cryptocotylar cotyledons.

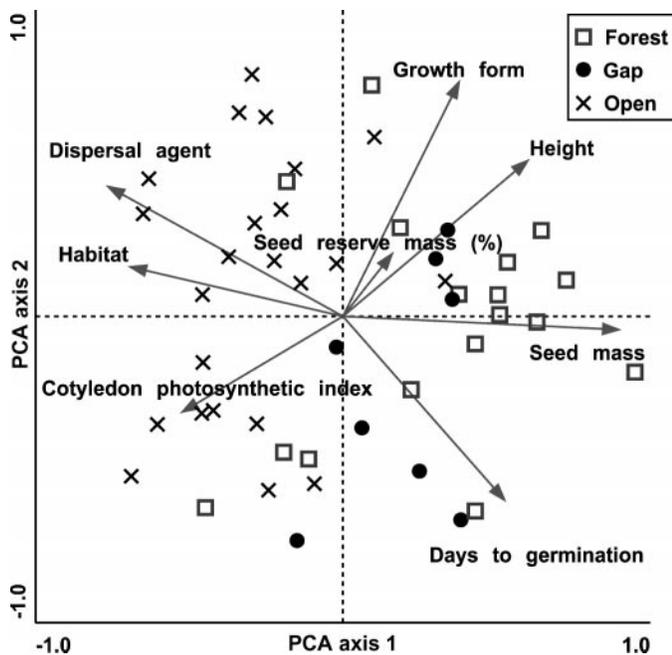|	Phylogeny not incorporated		Phylogeny incorporated	
Traits	Axis 1	Axis 2	Axis 1	Axis 2
Eigenvalue	3.7	1.9	4.4	2.0
Cumulative variance (%)	36.8	56.1	43.6	63.5
Total seed mass	**0.90**	−0.04	**−0.88**	−0.05
Dispersal agent	**−0.76**	0.42		
Habitat type	**−0.69**	0.16		
Adult height	**0.60**	**0.51**	−0.42	**−0.79**
Cotyledon photosynthetic index	**−0.52**	−0.31	**0.86**	−0.17
Growth form	0.38	**0.76**		
Days to germination	**0.53**	**−0.60**	**−0.59**	**0.53**
Seed mass comprised of seed reserve (%)	0.16	0.20	−0.38	−0.26

Fig. 3. Biplot of seed, seedling, and adult traits along axes 1 and 2 using principal components analyses without incorporating phylogeny for 48 tree and shrub species in Kibale National Park, Uganda. Discrete trait coding is as follows: habitat: forest = 1, gap = 2, open = 3. dispersal agent: large animal = 1, small animal = 2, nonanimal = 3. growth form: shrub/treelet = 1, tree = 2. Each point represents the position of the species scores in multivariate space, with symbols representing the habitat type of the species.

cotyledon reserves, is a more general reason why large seed size benefits seedling establishment and survival (Kitajima and Fenner, 2000; Westoby et al., 2002; Green and Juniper, 2004).

Overall, we found a suite of related traits anchored by seed size and cotyledon thickness. Species with large seeds, large seedlings, thick storage cotyledons, slow germination, large adult stature, and dispersal by large animals were associated with forest and gap habitats. Species with small seeds, small seedlings, thin photosynthetic cotyledons, fast germination, small adult stature, and dispersal by small animals were associated with open habitats. Many of these relationships are well supported in the literature (Garwood, 1996; Kitajima and Fenner, 2000; Ibarra-Manríquez et al., 2001), with the exception of the similarities found between forest- and gap-associated species. But because species germinating in one habitat often persist in the other habitat, a high degree of overlap in traits between these two habitats makes sense. Species found in these habitats also frequently overlap (Webb and Peart, 2000; Zanne and Chapman, in press).

Phylogeny—Based on phylogenetic signal tests, closely related species typically had similar cotyledon types, but did not have similar seed sizes. These results support earlier untested assertions that cotyledon morphology is phylogenetically more conservative than seed size (Garwood, 1996). Interestingly, results from the contingent change tests suggest that changes in cotyledon type are limited by the state of the dispersal agent and perhaps by seed size, but the reverse is not true. Thus, the evolution of different cotyledon types is likely dependent on the more labile seed size. In nonphylogenetic analyses, associations between cotyledon type and habitat type were weaker

than associations between seed size and habitat type. But in univariate and multivariate analyses in which phylogeny was incorporated, the strength of the relationships increased; in fact, cotyledon type is almost as equally important as seed size among the suite of traits that define PCA axis 1. These results suggest that a suite of life-history traits, especially incorporating cotyledon morphology, is not just associated with the species' current ecological distributions, but also changes in concert over evolutionary time.

Conclusions—Among 70 tree and shrub species in an East African forest, life-history traits varied together as a result of correlated evolutionary changes. The same suite of traits was identified in nonphylogenetic and phylogenetic analyses. Both seed size and cotyledon functional morphology were central in multivariate trait suites, especially in analyses that incorporated the phylogenetically conservative nature of cotyledon morphology. Finally, these traits were not only co-occurring but also interdependent, such that selection on one trait may lead to or limit change in other traits.

LITERATURE CITED

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