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Abstract

Seed size and toughness affect seed predators, and size-dependent investment in mechanical defence could affect relationships between seed size and predation. We tested how seed toughness and mechanical defence traits (tissue density and protective tissue content) are related to seed size among tropical forest species. Absolute toughness increased with seed size. However, smaller seeds had higher specific toughness both within and among species, with the smallest seeds requiring over 2000 times more energy per gram to break than the largest seeds. Investment in mechanical defence traits varied widely but independently of the toughness-mass allometry. Instead, a physical scaling relationship confers a toughness advantage on small seeds independent of selection on defence traits and without a direct cost. This scaling relationship may contribute to seed size diversity by decreasing fitness differences among large and small seeds. Allometric scaling of toughness reconciles predictions and conflicting empirical relationships between seed size and predation.

Keywords

Allometric scaling, functional traits, intraspecific variation, optimal foraging, seed defence, seed predation.

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INTRODUCTION

Seed mass varies over 11 orders of magnitude globally and 5–6 orders of magnitude within communities (Mazer 1989; Moles *et al.* 2003, 2005). Seed size underlies a key axis of variation in plant strategies (Westoby *et al.* 2002), and substantial research effort has focused on the evolutionary influences on, and ecological implications of, variation in seed size (Leishman *et al.* 2000). One important consequence of seed size is its influence on seed predation. Small seeds are hypothesised to experience lower predation due to several mutually compatible relative advantages; small seeds are less apparent, incorporate into soil more quickly, and assuming equal handling time, are relatively less efficient for seed predators (reviewed in Moles *et al.* 2003). Several studies show that seed predators prefer larger seeds of individual plant species (e.g. Brewer 2001; Jansen *et al.* 2002; Celis-Diez *et al.* 2004; Gómez 2004). Similarly, rodent exclosures favour recruitment of large-seeded species relative to small-seeded species (Reader 1993; Vaz Ferreira *et al.* 2011; Maron *et al.* 2012). However, interspecific tests for a relationship between seed size and predation show mixed results, with no relationship in a global meta-analysis (Moles *et al.* 2003) and contrasting patterns among habitat types in a second meta-analysis (Radtke 2011). Traits correlated with seed size within or among species may help explain these inconsistent results.

Seed predators are also sensitive to defensive traits, and mechanical defences of seeds can reduce predation. Tougher seeds can be exploited by fewer species due to maximum force constraints and are also more energetically costly per unit of reward than weaker seeds. Empirical evidence for toughness-dependent predation includes interspecific tests showing that predators including primates, ants, birds and rodents prefer soft-seeded species (Kinzey & Norconk 1990; Blate *et al.*

1998; Rodgerson 1998; Zhang & Zhang 2008). Similarly, predation is higher for softer seeds than for harder seeds in intraspecific comparisons (van der Meij & Bout 2000; Oliveras *et al.* 2008). Both seed size and seed defences influence seed predation.

A systematic relationship between seed size and mechanical defence would complicate any relationship between seed size and seed predation. If smaller seeds were relatively better defended – requiring more energy to consume per unit energy gained – the prediction for greater predation among large seeds would be reinforced. If larger seeds were relatively better defended, this prediction would be weakened, which might explain the lack of a strong interspecific relationship between predation and seed size. Mechanical defences that confer toughness, such as protective tissues or high tissue density, may be related to seed size. A global analysis found no relationship between protective tissue and seed mass (Moles *et al.* 2003). Community-scale analyses found negative relationships between seed size and seed coat thickness among congeners (Tiansawat *et al.* 2014) and between seed size and seed tissue density for a tropical forest (Wright *et al.* 2007). Alternatively, physical properties could underlie a relationship between toughness and seed size because objects become relatively weaker as they become larger (Bazant & Chen 1997). Scaling of seed toughness may make small seeds, relative to their size, tougher by a mechanism that is independent of natural selection on defence traits. To the best of our knowledge, a physical scaling relationship has not been invoked when considering the relative defences of large and small seeds.

We measured seed toughness and defence traits for 70 plant species at Barro Colorado Island, Panama. To assess relationships within and among species between seed size and mechanical defence, we assessed seed mass, protective tissue content, tissue density and toughness. Our analysis covers

relatively large-seeded species but spans four orders of magnitude in seed mass.

MATERIAL AND METHODS

We studied mechanical defence traits for 70 tree, shrub and liana species from the tropical moist forests of Barro Colorado Island, Panama (9° 9' N, 79° 51' W). We present data on protective tissue content, tissue density and toughness for fruits and diaspores (or 'seeds').

Protective tissue

To quantify protective tissue content, we used data from fruit dissections, described in detail by Wright *et al.* (2010). The dissection data include fresh and dry mass for fruits, diaspores, seeds (embryo and endosperm) and testa. Protective tissue content, also called physical protection, is calculated for diaspores as the dry mass of the diaspore minus the dry mass of the seed, divided by dry diaspore mass (Beckman & Muller-Landau 2011). This measures the mass portion of the diaspore that is protective tissue.

Fruit tissues, particularly exocarp, can also protect seeds within fruits, and our analysis includes fruits that confer mechanical defence. We exclude fleshy fruits that lack a tough exocarp (only diaspores were included for these species), and for our purposes assume that fruit pulp does not confer mechanical defence. We extend the measurement of protective tissue to fruits by taking the dry mass of the fruit minus the dry mass of all diaspores (comprising embryo, endosperm and diaspore protective tissues), divided by the dry mass of the fruit. In instances where the masses of all diaspores from a fruit were not measured, we multiply the average mass of the measured diaspores by the number of diaspores to estimate the mass of all diaspores. Because dissections did not separate flesh that provides a reward for seed dispersers, this approach may overestimate protective tissue content for fleshy fruits. To assess whether this influences our conclusions, we repeated analyses after excluding fleshy fruits and found qualitatively equivalent results.

Tissue density

We estimated tissue density for the same diaspore or fruit samples that were used in toughness analyses, described in detail below. For all samples, we recorded fresh mass, length, width and height. We divided mass by ellipsoid volume estimated using dimensional data (Wright *et al.* 2007) to obtain tissue density. This approach misrepresents tissue density when species differ from an ellipsoid shape. We avoid this potential problem because our analysis focuses on relationships within groups of samples defined by combinations of species and sample type (diaspore or fruit). Each 'species-by-sample type combination' is listed in Table S1. For a given species-by-sample type combination, samples will differ from an ellipsoid shape in a similar way. Although this may bias estimates of tissue density, it will not affect the slope of relationships between tissue density and other variables to be investigated here.

Toughness

We used a force testing machine to measure toughness. Samples were compressed between flat metal plates that moved together at a constant speed of 4.7 mm per min. We measured force (Newtons) and displacement (metres) with a load cell and an optical encoder (E5-720, US Digital, Vancouver, WA, USA) respectively. For smaller samples, we used a load cell that measures forces up to 1000 N to the nearest 0.5 N, and for larger samples, we used a load cell that measures forces up to 22,000 N to the nearest 5 N (BBS-250 and BBS-5k, Transcell Technology, Buffalo Grove, IL, USA). We used these data to assess the point at which samples broke. Break points coincided with a decrease in force as displacement increased. Because force sometimes decreased due to small fractures in the sample or repositioning of the sample as the load increased, we visually inspected the force–displacement curves to avoid misattribution of the break. For some samples, the break did not cause a decrease in force, and we determined the break point as the inflection point in the force–displacement curve. Repeating our analyses after excluding these samples did not affect our conclusions. Samples that otherwise did not have a discernable break were excluded from analyses. To obtain toughness, we integrated the area under the force–displacement curve up to the break point (Rodgers 1998). We express absolute toughness in terms of joules ($J = \text{kg m}^2 \text{s}^{-2} = \text{N m}$), the energy required to break the sample. We also present peak force (N), which is the maximum force exerted on the sample before it broke. As corresponding relative measures, we also present specific toughness (J/g) and specific peak force (N/g).

Analyses

We used linear mixed-effects models to analyse how protective tissue content, tissue density, peak force and toughness are each related to sample mass. In separate analyses, the response variables were protective tissue content (logit-transformed; Warton & Hui 2011), tissue density, peak force (log-transformed) or toughness (log-transformed). In each analysis, the fixed effect was log-transformed fresh sample mass with random slopes and intercepts for each species-by-sample type combination. We used likelihood ratio tests against corresponding models lacking the fixed effect to assess statistical significance.

Because we obtained tissue density, mass and toughness data from the same samples, we were also able to compare the influence on toughness of tissue density and sample mass together in a separate set of analyses. In the full model, the response variable was log-transformed toughness and the fixed effects were tissue density and mass, with random effects for slopes and intercepts for each species-by-sample type combination. Using the Akaike information criterion (AIC), we compared the full model and two nested models that included each fixed effect individually. We performed analyses with the R package lme4 (Bates *et al.* 2015).

RESULTS

We measured protective tissue content for 2678 fruit and diaspore samples and quantified both tissue density and toughness

for 434 samples. Our analysis covered 70 plant species from 58 genera and 24 families (Table S1), with samples ranging in mass between 0.01 g and 300 g. Toughness ranged widely in terms of the absolute energy required to break the sample (J) and relative to sample mass (specific toughness; J/g). The greatest specific toughness, exhibited in one of the smallest samples, was 2340 times greater than the lowest specific toughness, measured for one of the largest samples.

Protective tissue content did not vary consistently with sample mass (Fig. 1a). Although there were positive and negative relationships between mass and protective tissue content for individual species-by-sample type combinations (Fig. 1b), the overall relationship was non-significant (-0.11 ± 0.20 , estimated slope ± 1 SE; likelihood ratio test $P = 0.59$). The highest tissue densities recorded were among small samples across all species (Fig. 1c), but tissue density did not consistently have a positive or negative relationship with seed mass within species-by-sample type combinations (Fig. 1d; -0.05 ± 0.05 ; $P = 0.32$).

Absolute toughness was strongly related to mass (Fig. 2a; Table S1). We found a consistent negative allometry among and within species-by-sample type combinations (Fig. 2b; 0.71 ± 0.05 ; $P < 0.001$), indicating that specific toughness (J/g) strongly decreases as mass increases (Fig. 3a). This allometric slope was within one standard error of a $2/3$ allometry. The relationship between sample mass and peak force

(Fig. 2c) also had a negative allometry (Fig. 2d; 0.47 ± 0.05 ; $P < 0.001$).

Mass alone best explained absolute toughness. A model with mass as the single fixed effect outperformed the full model with mass and density as fixed effects (Table 1).

DISCUSSION

We found a strong negative allometry between seed mass and toughness – the energy required to break the seed – within and among species in our analysis of 70 plant species from Barro Colorado Island, Panama (Fig. 2a and b). Although larger seeds took more energy to break, toughness did not scale directly with mass. Instead, the relationship between mass and toughness had an allometric slope of roughly two-thirds. This negative allometry indicates that toughness per unit mass – specific toughness – decreases with increasing mass (Fig. 3a and b). Some of the smallest seeds required over three orders of magnitude more energy per gram to break than did the largest seeds.

Selection on mechanical defence traits such as protective tissue content or tissue density could cause greater specific toughness of small seeds. However, differential investment in these traits did not explain the consistent negative toughness-mass allometry observed within and among species. Protective

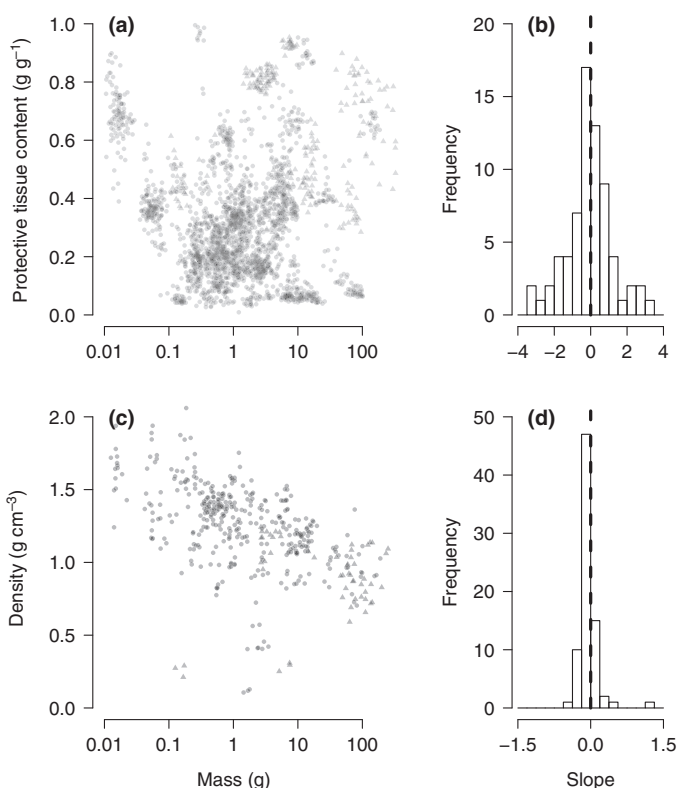


Figure 1 Relationship between fresh mass and protective tissue content (a) and tissue density (c). Point shapes indicate sample type, either diaspores (circles) or fruits (triangles). Histograms show slopes of the relationship between mass and protective tissue content (b) or tissue density (d) for each species-by-sample type combination.

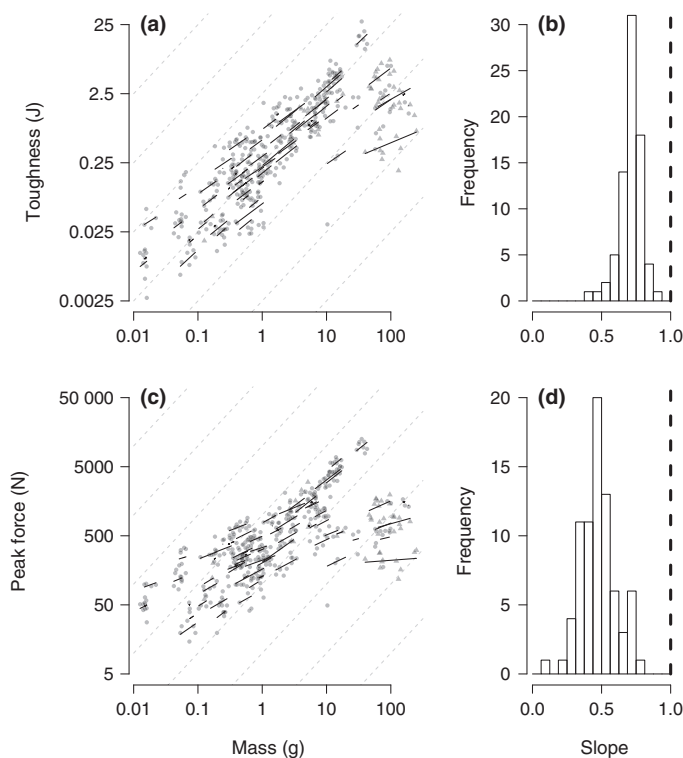


Figure 2 Relationship between diaspore (circles) or fruit (triangles) mass and toughness (a) and peak force (c). Lines indicate model fits for this relationship for each species-by-sample type combination. Grey dashed lines show an allometric slope of 1 for reference. Histogram of slopes for each species-by-sample type combination for mass-toughness relationships (b) or mass-peak force relationships (d), with values < 1 indicating a negative allometry, or decreasing toughness or force per unit mass as mass increases.

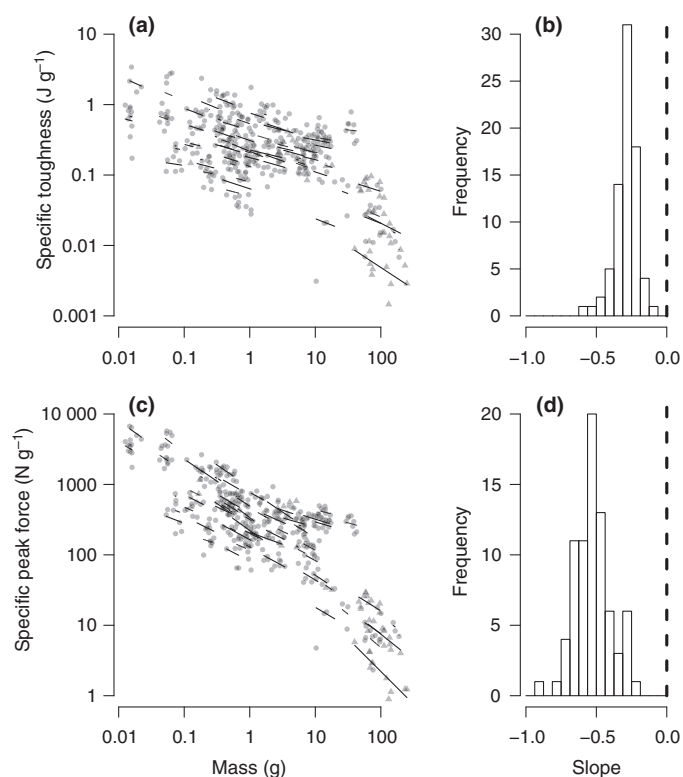


Figure 3 Relationship between diaspore (circles) or fruit (triangles) mass and specific toughness (a) and specific peak force (c). Lines indicate model fits for this relationship for each species-by-sample type combination. Histogram of slopes for each species-by-sample type combination for mass-specific toughness relationships (b) or mass-specific peak force relationships (d).

Table 1 Summary of three linear mixed models used to describe log-transformed toughness, with models ordered by increasing AIC. Models include log-transformed mass (g), tissue density (g/cm^3) or both as independent variables.

	Independent variables	Estimate \pm SE	<i>t</i> -value	AIC
1	Mass	0.708 \pm 0.048	14.7	69.6
2	Mass	0.696 \pm 0.049	14.1	72.9
	Density	-0.113 \pm 0.083	-1.37	
3	Density	-0.209 \pm 0.096	-2.17	162.9

tissue content was not consistently greater among small seeds (Fig. 1a and b), consistent with existing interspecific findings (Moles *et al.* 2003). Although tissue density was greater among small-seeded species (Fig. 1c), tissue density was unrelated to seed mass within species (Fig. 1d) and thus did not account for the negative toughness-seed mass allometry observed within species (Fig. 2a and b). We conclude that physical scaling of toughness (Bazant & Chen 1997) best explains this mechanical defence advantage of small seeds. Operating independently of selection on other defensive traits, this physical scaling relationship gives an advantage to small seeds that does not have a direct cost.

Our results contribute another mutually compatible mechanism predicting greater predation of large seeds. The

hypothesised advantages of small seeds for seed predation have been attributed to their lower visibility, faster incorporation into soil or lower handling time efficiency (Moles *et al.* 2003). The preferences for large seeds in experiments that offer seeds to captive seed predators cannot be explained by greater visibility or slower incorporation into soil. The relative toughness and lower handling time efficiency of small seeds are appropriate, and mutually compatible, explanations for the preference for large seeds in these experiments. Small seeds are also more likely to pass intact through mastication and the digestive tracts of ruminants and rodents (Janzen 1984; Williams *et al.* 2000; Shiels 2011). Greater toughness of small seeds appears to be the best explanation for greater survival probability of seeds that have already been discovered and handled.

Allometric scaling may reconcile the lack of an interspecific relationship between seed size and seed predation despite predictions for a positive relationship (Moles *et al.* 2003), and reports of greater predation of larger seeds by individual predator species (Brewer 2001; Jansen *et al.* 2002; Celis-Diez *et al.* 2004; Gómez 2004) or by similarly sized predators (Reader 1993; Ferreira *et al.* 2011; Maron *et al.* 2012). We found that an exponent of roughly two-thirds defined the power law relating toughness to seed mass (Fig. 2b). This suggests toughness is proportional to cross-sectional area while mass is proportional to volume. Muscle strength is also proportional to cross-sectional area, and smaller seed predators should be able to exert greater forces relative to their body mass. Thus, smaller seed predators should be less sensitive to the relatively greater toughness of small seeds. Similar scaling of both seeds and predators can therefore help explain why interspecific studies lack a strong relationship between seed size and predation, but studies on single predator species or similarly sized predators do show preferences for larger seeds.

The allometric scaling of seed toughness also has implications for the evolutionary ecology of seed size and defence and for the maintenance of seed size diversity. Plants face a trade-off between producing small seeds that are relatively tough but can be handled by a greater diversity of predators and producing large seeds that are relatively weak but can be handled by fewer predators. This trade-off and other familiar seed size-related trade-offs (e.g. seed number vs. size; seed reserves vs. size) shape seed size evolution. The scaling of seed toughness is likely to influence how selection acts on other components of plant defence in relation to seed size. For example, greater relative toughness of small seeds may weaken selection on chemical defence in small seeds relative to large seeds. The agents and severity of seed predation, persistence in the seed bank and interactions with frugivores are all likely to affect selection on seed toughness, and thus influence the evolution of seed size and defence. The scaling of seed toughness may also contribute to the maintenance of seed size diversity. Large seeds are generally considered more tolerant of stress (Muller-Landau 2010), but the source of biotic stress tolerance we demonstrate – the mechanical defence advantage of small seeds – may reduce the fitness differences between large and small seeds. This reduction in fitness differences could broaden seed size diversity within and among species. Thus, physical scaling of seed toughness may contribute to the impressive diversity of seed size observed globally.

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AUTHORSHIP

ECF and SJW designed the study. ECF analysed data and wrote the first draft of the manuscript. Both authors revised the manuscript.

REFERENCES

- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.*, 67, 1–48.
- Bazant, Z.P. & Chen, E.P. (1997). Scaling of structural failure. *Appl. Mech. Rev.*, 50, 593–627.
- Beckman, N.G. & Muller-Landau, H.C. (2011). Linking fruit traits to variation in predispersal vertebrate seed predation, insect seed predation, and pathogen attack. *Ecology*, 92, 2131–2140.
- Blate, G.M., Peart, D.R. & Leighton, M. (1998). Post-dispersal predation on isolated seeds: a comparative study of 40 tree species in a Southeast Asian rainforest. *Oikos*, 82, 522–538.
- Brewer, S.W. (2001). Predation and dispersal of large and small seeds of a tropical palm. *Oikos*, 92, 245–255.
- Celis-Diez, J.L., Bustamante, R.O. & Vásquez, R.A. (2004). Assessing frequency-dependent seed size selection: a field experiment. *Biol. J. Linn. Soc.*, 81, 307–312.
- Gómez, J.M. (2004). Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution*, 58, 71–80.
- Jansen, P.A., Bartholomeus, M., Bongers, F., Elzinga, J.A., Ouden, J.D. & Van Wieren, S.E. (2002). The role of seed size in dispersal by a scatter-hoarding rodent. In *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation*. (eds Levey, D.J., Silva, W.R., Galetti, M.). CABI Publishing, New York, NY, pp. 209–225.
- Janzen, D.H. (1984). Dispersal of small seeds by big herbivores: foliage is the fruit. *Am. Nat.*, 123, 338–353.
- Kinzey, W.G. & Norconk, M.A. (1990). Hardness as a basis of fruit choice in two sympatric primates. *Am. J. Phys. Anthropol.*, 81, 5–15.
- Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. (2000). The evolutionary ecology of seed size. In: *Seeds: the ecology of Regeneration in Plant Communities* (ed Fenner, M.). CABI Publishing, Wallingford, UK, p. 410.
- Maron, J.L., Pearson, D.E., Potter, T. & Ortega, Y.K. (2012). Seed size and provenance mediate the joint effects of disturbance and seed predation on community assembly. *J. Ecol.*, 100, 1492–1500.
- Mazer, S.J. (1989). Ecological, taxonomic, and life history correlates of seed mass among Indiana dune angiosperms. *Ecol. Monogr.*, 59, 153.
- van der Meij, M. & Bout, R.G. (2000). Seed selection in the Java sparrow (*Padda oryzivora*): preference and mechanical constraint. *Can. J. Zool.*, 78, 1668–1673.
- Moles, A.T., Warton, D.I. & Westoby, M. (2003). Do small-seeded species have higher survival through seed predation than large-seeded species? *Ecology*, 84, 3148–3161.
- Moles, A., Ackerly, D., Webb, C. & Tweddle, J. (2005). A brief history of seed size. *Science*, 307, 576–580.
- Muller-Landau, H.C. (2010). The tolerance-fecundity trade-off and the maintenance of diversity in seed size. *Proc. Natl Acad. Sci. USA*, 107, 4242–4247.
- Oliveras, J., Gómez, C., Bas, J.M. & Espadaler, X. (2008). Mechanical defence in seeds to avoid predation by a granivorous ant. *Naturwissenschaften*, 95, 501–506.
- Radtke, T.M. (2011). Granivore seed-size preferences. *Seed Sci. Res.*, 21, 81–83.
- Reader, R.J. (1993). Control of seedling emergence by ground cover and seed predation in relation to seed size for some old-field species. *J. Ecol.*, 81, 169–175.
- Rodgers, L. (1998). Mechanical defense in seeds adapted for ant dispersal. *Ecology*, 79, 1669–1677.
- Shiels, A.B. (2011). Frugivory by introduced black rats (*Rattus rattus*) promotes seed dispersal of invasive plant seeds. *Biol. Invas.*, 13, 781–792.
- Tiansawat, P., Davis, A.S., Berhow, M.A., Zalamea, P.C. & Dalling, J.W. (2014). Investment in seed physical defense is associated with species' light requirement for regeneration and seed persistence: evidence from *Macaranga* species in Borneo. *PLoS ONE*, 9, e99691.
- Vaz Ferreira, A., Bruna, E.M. & Vasconcelos, H.L. (2011). Seed predators limit plant recruitment in Neotropical savannas. *Oikos*, 120, 1013–1022.
- Warton, D.I. & Hui, F. (2011). The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, 92, 3–10.
- Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Evol. Syst.*, 33, 125–159.
- Williams, P.A., Karl, B.J., Bannister, P. & Lee, W.G. (2000). Small mammals as potential seed dispersers in New Zealand. *Austral Ecol.*, 25, 523–532.
- Wright, I.J., Ackerly, D.D., Bongers, F., Harms, K.E., Ibarra-Manriquez, G., Martínez-Ramos, M. *et al.* (2007). Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests. *Ann. Bot. London*, 99, 1003–1015.
- Wright, S.J., Kitajima, K., Kraft, N.J.B., Reich, P.B., Wright, I.J., Bunker, D.E. *et al.* (2010). Functional traits and the growth–mortality trade-off in tropical trees. *Ecology*, 91, 3664–3674.
- Zhang, H. & Zhang, Z. (2008). Endocarp thickness affects seed removal speed by small rodents in a warm-temperate broad-leaved deciduous forest, China. *Acta Oecologica*, 34, 285–293.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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