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Post-dispersal seed predation and its relations with seed traits: a thirty-species-comparative study

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Abstract

Post-dispersal seed predation is a key process determining the variability in seed survival in forests, where most seeds are handled by rodents. Seed predation is thought to affect seedling regeneration, colonization ability and spatial distribution of plants. Basic seed traits are the essential factors affecting rodent foraging preferences and thus seed survival and seedling recruitment. Many studies have discussed several seed traits and their effects upon seed predation by rodents. However, the results of those previous studies are usually equivocal, likely because few seed traits and/or plant species tend to be incorporated into these studies. In order to elucidate the relationships between seed predation and seed traits, we surveyed the predation of 48 600 seeds in a natural pine forest, belonging to 30 species, for three consecutive years. The results demonstrated that: (i) seed size and seed coat hardness did not significantly affect seed predation; (ii) total phenolics had a negative effect upon seed predation; (iii) positive effects of nitrogen content upon seed predation were found. From our study, it seems that the better strategy to prevent heavy predation is for plants to produce seeds with higher total phenolics content rather than physical defenses (i.e. hard seed coat) or larger seeds. Additionally, rodent foraging preference may depend more on Nitrogen content than other nutrient content of seeds.

Keywords: protein, rodent, seed predation, seed trait, total phenolics.

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Introduction

Seed predation by rodents, particularly after dispersal, is one of the most important processes of plant reproduction (Gill & Marks 1991). Post-dispersal seed predation, that is, predation following initial dispersal from the mother plant may affect seedling regeneration, colonization ability, and spatial distribution of the plants (De Steven 1991; Myster & Pickett 1993; Hulme 1996; Jansen *et al*. 2004; Vander Wall *et al*. 2006). Many factors may influence the extent of seed predation, including community vegetation structure and composition, abundance of seed predators, seed density and frequency, and seed traits (Hulme 1997; Ostfeld *et al*. 1997; Blate *et al*. 1998; Kollmann *et al*. 1998; Hulme & Borelli 1999; Hulme & Hunt 1999; Jones *et al*. 2003; Xiao *et al*. 2006; Wang & Chen 2009).

Seed traits, which can be seed quality indicators, are thought to have significant effects on rodent foraging preference and thus the seed fate itself (Kollmann *et al*. 1998; Hulme & Hunt 1999; Moles & Drake 1999; Jones *et al*. 2003; Moles *et al*. 2003; Xiao *et al*. 2006; Wang & Chen 2009). Most current studies have primarily focused on the effects of seed size on seed predation and the results are usually equivocal (Blate *et al*. 1998; Kollmann *et al*. 1998; Moles & Drake 1999; Moles *et al*. 2003; Xiao *et al*. 2006; Carrillo-Gavilan *et al*. 2010). For example, Moles *et al*. (2003) found a weak positive correlation between seed mass and the percentage of seeds remaining after 24 h of exposure to post-dispersal seed predators at two out of three field sites in Australia, but no significant relationship across 280 species from the global literature. However, effects of other morphological or chemical seed

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traits (e.g. fat content, protein content, tannin content, or hardness of seed coat, etc.), which vary widely among species, have been poorly studied. Based on 40 tree seed species, Blate *et al*. (1998) found that seed predation rates were negatively associated with seed size and the thickness and hardness of seed coat. Among 12 species, Kollmann *et al*. (1998) found no correlation between seed predation and seed protein content and that seeds with toxic components suffered lower predation. Most of the studies mentioned above found some correlations between seed traits and predation by rodents, but these relationships are not conclusive because few seed traits or plant species were considered.

In this study, 30 species (Table S1) of plant seeds were used to elucidate differences in seed predation among species and the relationships between seed predation and seed traits. Traits studied included: seed size (i.e. length, fresh mass, and dry mass), nutrient content (i.e. nitrogen, fat, starch, fiber, and ash), morphological defense (i.e. seed-coat hardness) and chemical defence (i.e. total phenolics). Considering that seed predation, and therefore the relationships between seed traits and seed predation, would differ among years, we conducted the research continually for three years to assess the temporal variance of rodent seed preference.

Materials and methods

Study area

This study was carried out in an alpine pine (*Pinus densata*) forest in the Shangri-La Alpine Botanical Garden, Hengduan Mountains, Yunnan province, southwestern China (27°54′ N, 99°38′ E, altitude 3456 m). The annual mean temperature is around 5.4°C, and annual rainfall is 625 mm. The forest is mostly primary growth with little human disturbance. *Pinus densata* is the dominant tree species, which coexists with several other tree and shrub species. The ground flora is covered by leaf litter (~1–10 cm in depth) and poorly developed, consisting of only a few herbs and mosses. For more details about the study site see Wang and Chen (2009). The fieldwork was conducted in October and November, being concordant with seeding phenology in the forest during three consecutive autumns (2004–2006). We did not survey the annual relative abundances of natural seed availability, however, no substantial differences in overall seed production were apparent according to our casual field observation.

Study species

Twelve native species were included in this study, and seeds of these species were collected directly from mother plants in the field; and in order to enlarge the sample size, 18 alien species of seeds bought from Seed and Seedling Company of Yunnan were also included. In total, there were 30 species of plant seeds used in this study: 7 species of seeds in 2004, 21 species in 2005, and 26 species in 2006 (Table S1). For the species producing fleshy fruits, all the fleshy parts wrapping the seeds were removed. All the experimental seeds were stored in the laboratory with normal temperature for less than 20 days, from being collected to being used in the experiment. The seed morphological measurements were based on 30 seeds of each species, including fresh mass (FM), dry mass (DM) (70°C, 72 h), length of seed, and dry mass of embryo-cum-endosperm fraction (EEF). Seeds were classified into one of three seed-coat-hardness categories (Blate *et al*. 1998): (i) soft, could be penetrated easily by a fingernail; (ii) hard, could not be penetrated by a fingernail; (iii) very hard, difficult to penetrate with a knife. We changed these categorized data into numerical data during the data analysis process (i.e. soft was 1, hard was 2, and very hard was 3). Seed chemical composition (i.e. fat, starch, fiber, and total phenolics) was preformed by the Center of Grain Quality of the Ministry of Agriculture, Kunming, China. Ash and nitrogen were measured by Global Chemistry Laboratory in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. We also calculated the chemical content of one seed by multiplying the proportion of each chemical composition by seed dry mass.

The sample for chemical analyses was about 200 g seeds for each species, including seed coats, because it was very difficult to decorticate the seed coats for many species. Total concentrations of N were determined by using an element autoanalyzer (Vario MAX CN C-N analyzer, Elementar Analysensysteme GmbH, Germany). Crude fiber was determined using an intermediate filtration method (Fibertec 2010, Foss, Denmark). Starch (amylose and amylopectin) was determined with the continuous flow method (AutoAnalyzer 3, SEAL Analytical GmbH, Germany). Crude fat was determined with the Soxhlet extractor method (Soxtec 2050, Foss, Denmark). Crude ash was determined by the dry-ashing method (550°C, LY/T 1268–1999). Total phenolics were then determined colorimetrically by spectrophotometric techniques (Visible Spectrophotometer SP-2100, Shanghai Spectrum). In accordance with some literature (Meng *et al*. 2012; Rehill & Schultz 2012) and our own experimental conditions, the Folin–Denis method was used to determine the total phenolics in the seed samples.

Seed deposition in field

Five parallel transects were set about 5 m apart in the forest and seeds were placed at 10 m intervals along each of the five transects. At each seed set station, we placed two plastic plates (12 cm in diameter) about 1 m apart with 15 seeds of the same species on each of them. Each species was replicated in five different seed stations. Throughout the transects, three, four, or five different species of seeds were set at any one time. We placed the seeds on the plates at 19:00 and checked at 07:00 the next morning for six consecutive days. During the 6 days, the data would be excluded if it rained at night, and continued to the next day. After each 07:00 seed check, seed fates were recorded, the residual seed fragments and the seeds that were ignored by the predators were removed, and new seeds were loaded onto the plates. In total, there were 7, 21, and 26 species of seeds included in 2004, 2005, and 2006, respectively (Table S1); and 900 seeds per year were loaded onto the plates for each species. The seed fates were sorted into two categories: (i) harvested, meaning the seeds were eaten (i.e. leaving only seed fragments on the plate) or removed by the rodents (i.e. seeds were absent from the plates and the area nearby the plates); (ii) ignored (i.e. intact seeds remaining on the plates or on the ground nearby the plates). The percent of seeds harvested during all the 6 days were used to estimate the seed predation extent. The assumption that seed removal equated directly with seed predation here may be a bit problematic because some seeds removed by rodents would be generally selected for storage (Jansen *et al*. 2004; Vander Wall *et al*. 2005). However, in our study area, seed removal could be assessed to be a reliable estimate of predation because almost all of the seeds cached by the rodents were recovered and consumed very soon afterwards (Wang *et al*. 2012).

We did not consider daytime seed predation or seed predation by birds or ants in this study because no seed predation was observed during the daytime and our previous study (Wang & Yang 2007) found ants and birds to contribute very little to overall seed predation.

Rodent survey

During the experimental time each year, live traps were baited with fresh peanuts to determine the key rodent species affecting seed predation. In order to minimize the trapping effect on the rodent population in the experimental plot where the seeds were released, the trapping plot was in the same forest about 500 m from the seed predation transects. Five transects were set, and ten trap stations at intervals of 5 m were set along each of the five transects for six consecutive days and nights. One live trap was set up at each trap station, and there were 300 traps for both day and night. Traps were checked twice daily (07:00 and 19:00 hours) and the captured rodents were recorded and taken to the laboratory. All the captured rodents were subsequently released at the sites where they were originally captured after the survey.

Data analysis

All the 30 species used in this study, including 12 native species and 18 alien ones, were treated as one dataset during the analysis processes for the following reasons: (i) introducing 18 alien species was in order to enlarge the sample size; (ii) the purpose of this study was to elucidate differences in seed predation among species and the relationships between seed predation and seed traits, but not the difference of seed predation between sources of seeds; (iii) it was difficult to run the statistical analysis if native species were separated from alien ones because of the limited sample size; (iv) some studies found that the familiarity to species may affect rodent seed selection (Pyare *et al*. 1993), however, no significant seed predation differences were found between native and alien species in our study; (v) it is believed that rodents in our study area did have enough ability to deal with the alien seeds, because our previous studies found that rodents could quickly detect fine distinctions of artificial seeds with different seed size, tannin and nutrient content, which they had never met before (Wang & Chen 2009, 2012).

SPSS for Windows (Version 13.0; IBM Corporation, Armonk, New York, USA) was used for conducting all the statistical analyses. Spearman's rank correlation coefficient was employed to estimate the correlations between seed predation and seed traits, because the seed predation data in 2006 did not follow a normal distribution. One-way ANOVA was used to test the differences of seed traits among the three categories of seeds suffering different predation. Considering that the correlations among variables may not be negligible, multiple regression was also employed to analyze the relationships between seed predation and seed traits. In order to reduce the number of independent variables and thereby explain variation in a few dimensions, principal component analysis (PCA) was preformed to evaluate the relationships among seed traits. Following PCA, Varimax orthogonal rotation was applied to construct a new and more easily interpretable pattern of component loadings. We only considered nine variables during the multiple regression and PCA analyses, because several seed traits were highly related to seed size (e.g. seed length, fresh mass, dry mass, and the chemical content of one seed). These nine variables were seed dry mass, EEF (%), fat, fiber, starch, N content, total phenolics, ash and water content. For the multiple regression and PCA analyses, predation data in 2006, dry mass and starch content had been normal transformed by using Blom's proportional estimation formula, as the original data did not follow a normal distribution.

Results

Dominant rodents

No predators were captured by day traps during the three experimental years. The night traps captured nine individuals in 2004 and eight in 2005; all of them were Sichuan field mouse (*Apodemus latronum*). Sixteen individuals were captured in 2006. Nine of them were *A. latronum* (56.3%), six were Chevrier's field mouse (*A. chevrieri*) (37.5%), and one was Southwest China vole (*Eothenomys custos*). All three species captured in the night traps were seed predators. *Apodemus* was the main predator in the study area.

Overall seed predation

There was a wide range of predation on all seed species during the three years of experimentation (5.3–99.7% in 2004, 0.2–94.0% in 2005, and 0.4–100% in 2006) (Fig. 1a). Seed predations were significantly different among years ($F = 8.852$, d.f. = 2, $P < 0.001$). Seeds suffered the highest predation in 2006 (70.7 ± 7.3%, mean ± SE) and lowest in 2005 (29.1 ± 6.4%, mean ± SE) (Fig. 1a). No significant seed predation differences were found between native and alien species both in 2005 (*t*-test = 0.806, d.f. = 19, $P = 0.430$) and 2006 (*t*-test = 1.658, d.f. = 24, $P = 0.110$), while only native species were tested in 2004. The 30 species were sorted into three categories according to their seed predation. Species suffered heavy predation during all experimental years (e.g. *Pinus armandii*) were sorted into category I, that is, preferred by predator; species that suffered little predation through the three years (e.g. *Iris bulleyana* and *Cotoneaster* sp.) were into category II, that is ignored by predator; while other species that suffered high predation in one year but not in other years (e.g. *Sinopodophylum hexandrum*, *Hippophae* sp., and *Bauhinia* sp.) were sorted into category III, that is, moderately preferred by predators (Fig. 1b). However, most species were not tested throughout the 3 years. Furthermore, it is logical that seeds suffering heavy predation in 2005 with the lowest mean predation rate (29.1%) could be sorted into category I, while seeds suffering little predation in 2006 with the highest mean predation rate (70.7%) could be sorted into category II. Based on the results above and the frequency distribution of seed predation in the 3 years (Fig. 1a), the three categories were divided as follows: (i) preferred by predators ($n = 6$), which included the species suffering more than 40% predation in 2005; (ii) ignored by predators ($n = 4$), which included the species suffering less than 10% predation in

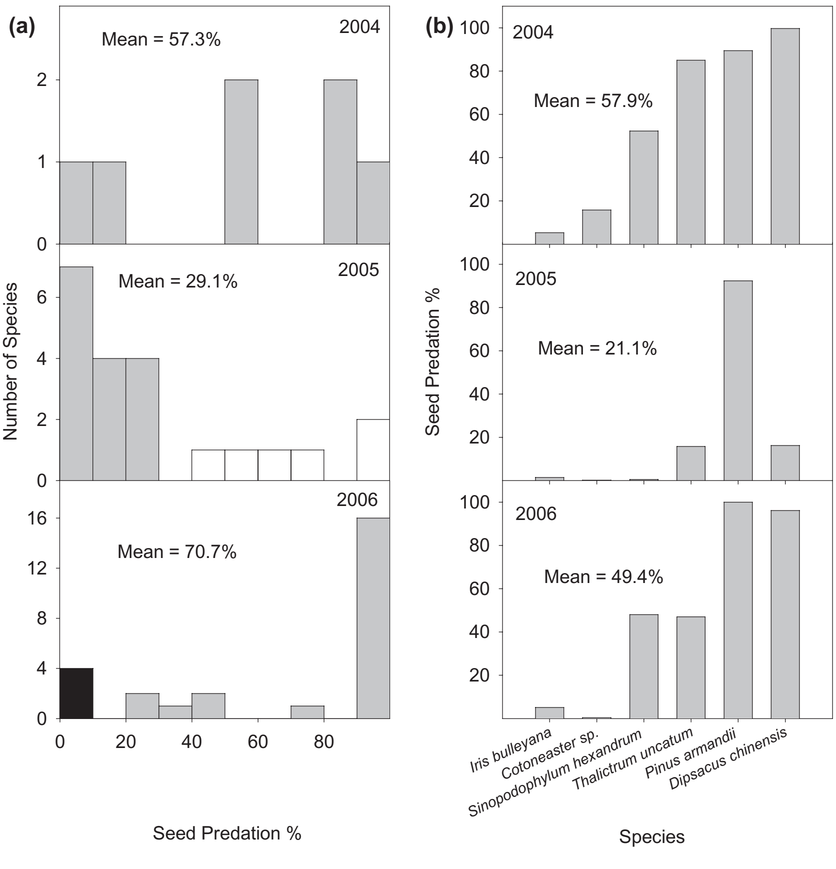

Fig. 1 Overall seed predation in the three years (mean value of seed predation ratio are shown). (a) The frequency distribution of seed predation ratio for each year. Open bars stand for preferred species of seeds by predators ($n = 6$), black bars stand for ignored species of seeds by predators ($n = 4$), and gray bars stand for moderately preferred species of seeds ($n = 20$). (b) Seed predations of the six species that were surveyed in all the three years.

2006; and (iii) moderately preferred by predators ($n = 20$), which included all other species (Fig. 1, Table S1).

Effects of seed traits upon seed predation

Spearman's rank correlation analyses indicated no significant relationship between seed predation (percentage of seed harvested by rodents) and seed size (including seed length, fresh weight, dry weight, and EEF) or seed chemical content in one seed ($P > 0.05$) in both 2005 and 2006. Statistical relationships were found between seed predation and a few nutrient contents. The results showed that seed predation was positively correlated with N ($r = 0.477$, $P < 0.05$) and ash content ($r = 0.443$, $P < 0.05$) in 2006, but not in 2005. Data of 2004 were not analyzed because of limited sample size.

Multiple regression analyses indicated no significant models could fit seed predation and the nine seed trait variables both in 2005 (adjusted $R^2 = -0.162$, $P = 0.706$) and 2006 (adjusted $R^2 = 0.200$, $P = 0.171$). Data of 2004 were not analyzed because of limited sample size.

ANOVA tests indicated significant differences among the three predation categories of seeds and proportion of EEF, N, ash, fiber, and total phenolics content only (Table 1). Total phenolics content of ignored seeds was significantly higher than of others (13.32% vs 2.38% and 4.58%). The N contents of both preferred seeds and ignored seeds were significantly lower than the moderately preferred seeds. Ash content of ignored seeds was significantly lower than the moderately preferred seeds, but not significantly lower than preferred seeds. Fiber content of preferred seeds was significantly higher than moderately preferred seeds but not higher than ignored seeds (Table 1).

The PCA results showed that the first three principal components accounted for 63.72% of the total variation in the seed traits of the 30 seed species (Table 2). The first axis of the PCA represented decreasing dry mass and water content, and increasing EEF, ash, and N content, explaining 25.91% of the variance. The second axis represented decreasing fiber content and increasing starch content and dry mass, explaining 20.54% of the variation. The third axis included parameters associated with total phenolics and fat content, and explained 17.28% of the variation (Table 2). No significant relationships were found between seed predation and the first two principal components but for PC3 there was ($r = 0.448$, $P < 0.05$): moderately preferred seeds had a significantly higher PC1 value (Table 1, Fig. 2).

Table 1 One-way ANOVA summaries of seed traits in relation to seed predation among different predation categories of seeds. Mean values (± 1 SE) are presented

Seed traits	Preferred ($n = 6$)	Moderately preferred ($n = 20$)	Ignored ($n = 4$)
Length (mm)	8.18 ± 1.17	6.58 ± 0.78	8.76 ± 2.17
FM (g)	8.78 ± 3.99	11.57 ± 7.15	4.66 ± 2.05
DM (g)	8.02 ± 3.67	10.85 ± 6.76	4.36 ± 1.94
Water (%)	7.79 ± 1.46	6.69 ± 0.28	6.52 ± 0.64
EEF (g)	3.20 ± 1.71	5.02 ± 2.25	2.65 ± 1.40
EEF (%)	41.82 ± 5.92^{a}	71.98 ± 4.64^{b}	64.48 ± 21.20^{ab}
N (%)	2.20 ± 0.33^{a}	3.65 ± 0.26^{b}	1.73 ± 0.47^{a}
Fat (%)	20.68 ± 4.59	19.97 ± 2.14	24.51 ± 9.35
Starch (%)	1.33 ± 0.39	4.12 ± 1.27	1.17 ± 0.48
Ash (%)	3.17 ± 0.73^{ab}	4,52 ± 0.33^{a}	2.32 ± 0.40^{b}
Fiber (%)	46.83 ± 3.06^{a}	26.12 ± 2.89^{b}	39.05 ± 10.46^{ab}
Total phenolics (%)	2.38 ± 0.88^{a}	4.58 ± 0.94^{a}	13.32 ± 7.39^{b}
Hardness	2.17 ± 0.40	1.75 ± 0.16	1.50 ± 0.50
n (mg/seed)	1.49 ± 0.68	2.80 ± 1.26	0.97 ± 0.68
Fat (mg/seed)	20.67 ± 9.44	21.06 ± 12.02	16.04 ± 12.29
Starch (mg/seed)	0.94 ± 0.54	6.96 ± 4.46	0.57 ± 0.37
Ash (mg/seed)	1.87 ± 0.69	4.17 ± 2.28	1.11 ± 0.59
Fiber (mg/seed)	39.60 ± 15.75	24.06 ± 15.91	17.48 ± 6.40
Total phenolics (mg/seed)	1.43 ± 0.56	3.57 ± 1.70	2.97 ± 1.66
PC1	–0.94 ± 0.48^{a}	0.40 ± 0.15^{b}	–0.61 ± 0.56^{a}
PC2	–0.39 ± 0.22	0.18 ± 0.24	–0.28 ± 051
PC3	0.27 ± 0.23	0.10 ± 0.19	–0.92 ± 0.091

Values followed by different letters indicate significant differences among different categories of seeds.

FM, fresh mass of 100 seeds; DM, dry mass of 100 seeds; EEF, embryo-cum-endosperm fraction of 100 seeds; PC, principal component.

Table 2 Principal component analyses for seed traits among the 30 seed species used in this experiment. Each factor represents an ordination axis. See text for definitions

Variable	Factors PC1	 PC2	 PC3
EEF	0.744	0.035	−0.314
Ash	0.722	−0.008	0.313
N	0.640	0.396	0.408
DM	−0.622	0.562	0.306
Water	−0.512	−0.247	0.009
Starch	0.026	0.850	−0.130
Fiber	−0.424	−0.763	0.142
Total phenolics	0.113	0.062	−0.867
Fat	0.073	−0.070	0.555
Eigenvalue	2.332	1.849	1.555
Variance explained (%)	25.910	20.539	17.275
Cumulative variance	25.910	46.449	63.724

DM, dry mass of 100 seeds; EEF, embryo-cum-endosperm fraction of 100 seeds.

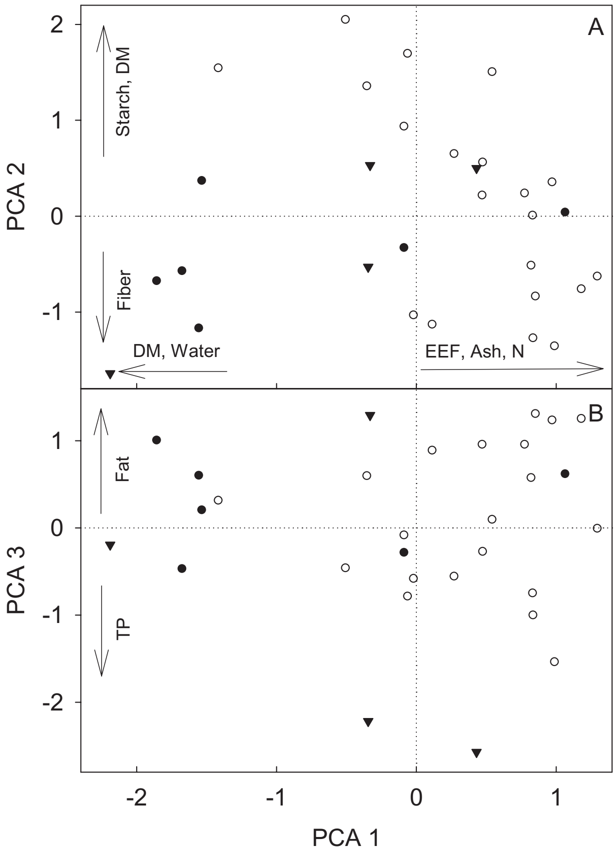

Fig. 2 Ordination of 30 seed species on the first three axes of a principal component analysis. Filled circles stand for preferred species of seeds by predators ($n = 6$), filled triangles stand for ignored species of seeds by predators ($n = 4$), and open circles stand for moderately preferred species of seeds ($n = 20$). EEF, embryo-cum-endosperm fraction of 100 seeds; TP, total phenolics.

Discussion

Seed size is a phenotypic trait, and it is often argued that larger seeds should be more susceptible to vertebrate predation (Kelrick *et al*. 1986; Willson & Whelan 1990; Boman & Casper 1995). However, no seed-size effects upon seed predation were found in our study, which contradicts many studies (Forget *et al*. 1998; Vander Wall 2003; Jansen *et al*. 2004; but see Blate *et al*. 1998; Kollmann *et al*. 1998; Moles *et al*. 2003). Logistically, rodents can get more energy by consuming larger seeds than smaller ones; however, manipulating larger seeds may result in longer time to reach the edible tissue, thus increasing the rodent's predation risk. Rodents must balance the seed size and predation risk when foraging. Intraspecies studies usually find significant effects of seed size on seed predation (Vander Wall 2003; Jansen *et al*. 2004). On the other hand, interspecies studies tend to find no such relationship (Blate *et al*. 1998; Kollmann *et al*. 1998; Moles *et al*. 2003; and this study). This may because all seed traits are often combined in experiments and other seed traits may interact with seed-size effects upon seed predation.

Total phenolics, especially tannins, as a series of important denfence components, are widely distributed among species of plant seeds (Shimada 2001; Smallwood *et al*. 2001; Steele *et al*. 2001; Wood 2005; Xiao *et al*. 2006; Wang & Chen 2008). In this study, twenty-nine of the 30 species contained phenolics (0.13–26.48%). The ANOVA tests and PCA both showed that seeds with higher total phenolics content suffered lower predation (Table 1, Fig. 2), however, these negative effects were not simply following linear correlations (Spearman's rank correlation coefficient, $P > 0.1$). These results concur with other studies on fewer species (Smallwood & Peters 1986; Smallwood *et al*. 2001; Wood 2005) and suggest high content of total phenolics/tannins might protect seeds from predation by rodents. It seems that, for the plants in the present study area, high total phenolics content is a better strategy than physical defenses (i.e. hard seed coat) against heavy predation (Table 1). However, such a conclusion is not straightforward. There were many alien species used in our study, thus the significant effect of phenolics may reflect that rodents were unfamiliar with many of the seeds and higher phenolics content caused an initial rejection of the seeds. Furthermore, many species of seeds in our study (50%) contained very little tannin (< 2%), while species with high tannin content (> 10%) were relatively few (17%). So, more high-tannin-content species, especially local species, should be included in future studies.

A positive correlation between N content and seed predation was found in our study, however, the ANOVA tests showed that the preferred seeds were not those with the highest N content (Table 1). It is usually accepted that N can be considered as a strong protein signal (Pastor-Cavada *et al*. 2010), but some other studies also found that rodents do not prefer the highest protein-content diet or seeds (Lewis *et al*. 2001; Bensaid *et al*. 2002; Takahashi & Shimada 2008). As important indicators of seed quality, fat, starch, ash, and also the percentage of EEF have usually been considered to play important role on seed predation by rodents (Kelrick & MacMahon 1985; Smallwood & Peters 1986; Vickery *et al*. 1994; Harrison *et al*. 2003; Caccia *et al*. 2006; Xiao *et al*. 2006; Wang & Chen 2012). Therefore, we had also expected rodent foraging preferences to be related to these important seed traits, but no such correlations were found. Kollmann *et al*. (1998) also failed to find such relations between seed predation and seed traits (e.g. seed size, N content, and percentage of EEF), and they discussed that the effects of these seed traits were obscured by the influence of differences in seed viability. Crude fiber, another important seed trait, can be considered as a "proxy trait" of seed coat (Hudson 1984; Mueller & van der Valk 2002). Many studies showed significant negative effects of seed coat (e.g. hardness and thickness) on seed predation (Janzen 1971; Blate *et al*. 1998; Xiao *et al*. 2006; Grant-Hoffman & Barboza 2010), however, no correlations between seed predation and fiber content were found in our study.

Current literature clearly shows that one of the most important factors affecting seed selection (for both consumption and hoarding) is the germination schedule and the resulting perishability of seeds (Smallwood *et al*. 2001; Steele *et al*. 2001). This one factor alone may be very important, at least in years in which natural, ambient food abundance is high. However, perishability and storability were not considered during the analyses in this study. We did not test the seed germination schedules, but we were sure that seeds from both local and alien species would require a long dormancy period for germination and suffer little nutrient loss for the following reasons: (i) few seedlings were found in the field during the experimental period, suggesting that most of the local species may require a long dormancy period for germination; (ii) logically, germination may be very difficult for both local and alien species because of the extremely low temperature and less rainfall during the experimental period; (iii) seeds in the field were found suffering little attack by insects or fungus (pers. obs.).

In our study, seed coats were included when we analyzed the chemical components of seeds because of the extreme difficulty of separating seed coats from seed kernels for most of the species. However, rodents usually do not ingest seeds with seed coats, and they only eat the edible kernels of seeds. Thus, it may be suspicious to examine the relationships between chemical traits and seed predation by rodents, because chemicals in seed coats may have no effects on rodents' foraging preference. Seed coats indeed contain some chemicals (Troszynska & Ciska 2002; Zhou *et al*. 2010), however, the dominant component of seed coats are fiber, and fiber is usually considered as a "proxy trait" of seed coat (Hudson 1984; Mueller & van der Valk 2002), which can be an important indicator of physical defense of seeds; while most chemical defenses and nutrients are contained in the seed kernels (Shimada 2001; Xiao *et al*. 2006; Takahashi & Shimada 2008).

In addition to this study, many studies have used a similar methodology of placing a population of seeds on the ground, singly or in clumps, and monitoring their predation and removal over time (Blate *et al*. 1998; Moles *et al*. 2003). Some other studies have suggested that seed removal cannot simply be considered equivalent to seed predation, because seeds removed by rodents are generally cached for later use and these stored seeds frequently survive to germination (Jansen *et al*. 2004; Vander Wall *et al*. 2005). The intention of removal by rodents is eventual seed predation, thus studies considered seed removal equivalent to predation could still provide some useful information on seed dynamics (e.g. escape from predation). In our study area, rodents often suffer food shortages during the winter, which exceeds five months, suggesting the need to store food to overcome such lean periods. However, our previous study suggested that seed removal could be assessed to be a reliable estimate of predation eventually because almost all of the seeds removed and cached by the rodents were recovered and eaten within a few days (Wang *et al*. 2012).

There were large annual variations in seed predation among years, even for the same species (Fig. 1), demonstrating that rodents may not be able to consistently assess the seed traits. Seed abundance was found to play a significant role on rodent foraging behavior (Vander Wall 2002; Jansen *et al*. 2004), but in our study area, no substantial differences in overall seed production were apparent from casual field observation. However, there was a great annual variation in rodent densities during our study (trap success: 3.0%, 2.7% and 5.3%. respectively). Several other studies have also found that spatial and temporal variation in rodent population fluctuation and seed, thus rodent abundances may be potential factors influencing the seed predation results (Jansen *et al*. 2004; Li & Zhang 2007).

In this study, we surveyed seed predation of 30 species for three consecutive years. Spearman's rank correlation coefficient, multiple regression, ANOVA test, and PCA were used to elucidate the relationships among seed predation and seed species and seed traits. However, only a few significant effects of seed traits on seed predation were found, and differences in results existed depending on statistical methods. One possible explanation may be that the effects of seed traits on rodent foraging behavior may not be discernable with simple linear correlations, because of a series of complex processes. Furthermore, another potential reason may be the limited sample size (i.e. only 30 species were considered in this study), resulting in limited variation of seed traits. For example, the contents of starch were much lower than previous reports in other forests, and this probably may be due to lacking several large seeded species such as *Quercus*, of which the starch content could reach more than 60% (Xiao *et al*. 2006).

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1 Summary of morphological and nutritional traits of 30 seed species. FM, fresh mass of 100 seeds; DM, dry mass of 100 seeds; EEF, embryo-cum-endosperm fraction of 100 seeds.