Teasing Apart the Effects of Seed Size and Energy Content on Rodent Scatter-Hoarding Behavior

Bo Wang1, Xiaolan Yang1,2

1 Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan province, China, 2 Ecotourism Faculty, Southwest Forestry University, Kunming, Yunnan province, China

Abstract

Scatter-hoarding rodents are known to play a crucial role in the seed dispersal of many plant species. Numerous studies have indicated that both seed size and the energy content of seeds can affect rodent foraging behavior. However, seed size is usually associated with energy content per seed, making it difficult to isolate how seed size and energy affect rodent foraging preferences. This study used 99 treatments of artificial seeds (11 seed sizes × 9 levels of energy content) to tease apart the effect of seed size and energy content on rodent seed-caching behavior. Both seed traits showed significant effects, but their details depended on the stage of the rodent foraging process. Seeds with higher energy content were harvested more rapidly while seed size only had a modest effect on harvest rate. However, after harvesting, seed size showed a much stronger effect on rodent foraging behavior. Rodents’ choice of which seeds to remove and cache, as well as seed dispersal distance, seemed to reflect an optimal seed size. Our findings could be adapted in future studies to gain a better understanding of scatter-hoarding rodent foraging behavior, and the co-evolutionary dynamics between plant seed production and seed dispersers.

Introduction

Scatter-hoarding rodents are known to play a crucial role in seed dispersal of many plant species, because they usually store large quantities of intact seeds in the soil at many caches [1–8]. When scatter-hoarding rodents encounter a seed, they usually face several sequential options: harvest vs. ignore the seed; if they choose to harvest, eat in situ or move it to elsewhere; upon removal, decide on the removal distance, and whether to eat or cache the removed seed [9]. Basic seed traits, such as seed size [4,10–12], seed geometry [13,14], chemical content [13,15–18], energy/nutrient content [5,12,19,20], and germination schedule [16,21], are believed to be essential factors during these important foraging processes.

Seed size is one of the most important seed traits, and its effects on rodent foraging behavior have been extensively discussed. Seed size is usually characterized by two measures – seed mass and seed volume – and both measures are usually positively correlated not only within a species but also among species [22,23]. Several studies have additionally incorporated seed volume parameters (e.g. area, length, etc.) and their effects on rodent seed foraging. Perea et al. found that animals usually preferred seeds with larger areas than the ones with smaller areas [24], while Holl and Lulow found that neither seed length nor seed weight were correlated with the proportion of seeds predated [22]. However, the majority of the current literature tends to use seed mass. For example, Blate et al. found a significantly negative relationship between seed harvest and seed mass [25], though this was not supported in other studies [15,26]. Moles et al. found a weakly negative correlation between seed mass and the proportion of seeds harvested at two of their three field sites in Australia, but no significant relationship across 280 species from the global literature [27]. A number of studies have found that heavier seeds were more likely to be removed rather than eaten in situ [4,5,10,28,29], while others found medium-massed seeds were more commonly removed than either lighter or heavier seeds [30]. Seed size was also positively correlated to the distance of seed removal by rodents [19,31,32].

Why does seed size play such an important role on rodent foraging behavior? First, seed size is usually positively correlated with energy content [4,12,19], and rodents usually prefer food of higher energy content even when their sizes are the same [12,20,33]. Xiao et al. found that the seed energy density – energy per unit weight – plays a key role in rodent foraging decision making process; small Camellia oleifera seeds (0.47 g) were harvested as quickly and ultimately the same proportions was removed as large Lithocarpus harlandii seeds (3.14 g) [5]. Notably, the smaller C. oleifera seeds contained much more fat (51.79% vs. 0.91%) and energy (29.56 vs. 17.11 kJ/g) per unit seed weight than L. harlandii [5]. On the other hand, though energy content tends to rise with seed size, larger seeds require more handling time [9,11,34]. As such, an optimum seed size may exist that trades off energetic benefits against time/handling costs. For example, rodents preferred to harvest and cache more medium-sized seeds than big and small seeds [9]. Jansen et al. found that for Carapa procera, the maximum removal distance for caching was...
associated with seeds about 29 g, and that dispersal distance decreased with greater seed weight [35].

As discussed above, both seed size and energy content of seed influence scatter-hoarding rodent foraging preferences. However, it is difficult to detect which of the two, seed size or energy content, contributes more, especially across species. Using artificial seeds we can experimentally test the effect of a univariate trait, while keeping other traits constant [9,12,20,33,36]. For example, Wang et al. used artificial seeds made from clay and peanut powder to investigate foraging preference of scatter-hoarding rodents, and their results showed that seed size affected the probability of a seed being harvested, removed, and cached as well as the seed dispersal distance, while energy content only affected the probability of seed harvest and dispersal distance [9]. However, their experiment only targeted the effects of seed size and energy content separately without their interactions, which might play an important role.

Wang and Chen used similar artificial seeds and tried to test the interactive effects of both seed size and energy content on the rodent foraging preference, and their results indicated that seed size is a decisive factor which might attenuate the effects of energy content [12]. However, their experimental designs contained only three seed sizes and two levels of energy content [12], thus a comprehensive understanding of the interactions may not have been obtained.

To further explore how seed size and energy content affect scatter-hoarding rodent foraging preferences, we manipulated seed size and energy content levels by using an artificial seed system [12]. In this study, we created 99 different treatments of artificial seeds with 11 seed sizes, with each seed size having 9 levels of energy content. Our objective was to evaluate the effects of seed size and energy content separately, and also their interactions. Based on the literature and our previous findings, we predict that, (1) rodents will show an increasing preference for higher energy content throughout the entire scatter-hoarding process (i.e. seed harvest, removal, caching and the dispersal distance), as rodents can increase their net rate or efficiency of foraging by choosing higher energy content food; (2) rodents will prefer an optimum seed size (i.e. medium size) during the scatter-hoarding process, as profitability incorporates the tradeoff between handling time and energy content of a seed, both of which scale positively with seed size; (3) compared to energy content, seed size will have a stronger effect on seed removal and dispersal distance, as transporting seeds and subsequently caching them logically require more time and energy than the initial harvest stage, and seed size usually has a direct bearing on handling time and energy cost.

Materials and Methods
Ethics Statement
This study was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of China. The protocol was approved by the Administrative Panel on the Ethics of Animal Experiments of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (Permit Number: XTBG2012-006). We signed a contract (No. 20120015) with the Shangri-La Alpine Botanical Garden in 2012 permitting access to the study site for conducting the experiments.

Study site
The experiment was carried out in October 2012, in a natural forest in the Shangri-La Alpine Botanical Garden, Yunnan province, southwestern China (27°34’ N, 99°38’ E, altitude 3456 m). Here Pinus densata is the dominant tree species, and it coexists with several other tree species, e.g., Pinus armandi, Betula delavayi, Picea brachytyla, and Populus spp. Sichuan field mouse (Apodemus latronum) and Chevrier’s field mouse (Apodemus chevrieri) were the two most abundant seed predators/dispersers in the forest; few other animals were found eating or removing the artificial seeds [12,20,37]. Apodemus latronum was slightly more common than A. chevrieri according to our live-trapping census (trap success: 6.7% vs. 1.2%, B. Wang and X. Yang, unpublished data). Both rodent species have similar body sizes, about 10 cm in length (without the tail) and only tens of grams in weight. Our previous studies found that both species in the field exhibited similar foraging behavior toward the artificial seeds as they would toward natural seeds in terms of consumption, carrying, and caching [12,23].

Study materials-Artificial seeds
We used clay and peanut powder to produce artificial seeds (Fig. S1). Both clay and peanut powder were ground in a mortar until it passed through a 1-mm screen. The resulting powder was mixed and homogenized thoroughly, and then water was added to make a consistent dough. This dough was used to make ball-shaped artificial seeds. A 15-cm thin steel thread with a spiral hook on one end was embedded into each seed and connected to a small red plastic tag (2.5 cm in length and 0.7 cm in width) on the other end. Each tag was numbered for seed identification as well as for detecting where the cached seeds were. After natural drying and hardening, the tags were attached strongly. No tags were found fallen off during the experiments. The length of the steel thread was sufficient to detect the cached seeds since rodents in our study site usually cached the seeds in depths less than 2 cm in the soil, leaving the tags on the surface easy to be detected. For more details see Wang and Chen [12].

Experimental design
We collected seeds of 11 common plant species in our study forest for analysis to determine the variation in seed size. The mean seed length was 0.8±0.1 cm, with a very narrow range of 0.4–1.4 cm (Table S1). Thus, in order to get a much clearer pattern about rodent foraging preference upon seed size, we used similar experimental design as our previous study [9], in that we expanded the range of artificial seed size (0.2–4.0 cm) beyond what was naturally observed. We devised 99 different treatments of artificial seeds with 11 seed sizes (0.2, 0.4, 0.6, 0.9, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 cm in diameter) each having 9 levels of energy content (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of peanut powder in weight). The diameter of seeds was used as the measure of seed size in our study. Furthermore, clay is denser than peanut powder so that the percentage of one over the other within a seed would change the overall seed mass but not the volume since the diameter was controlled.

Five plots (2 m×2 m) separated by more than 50 m were established. At each plot, we located 9 seed release points in a 3×3 rectangular grid, with about 1 m between points. Each circle-shaped point was about 15 cm in radius, and the seeds were placed along the circle with the tags pointed outwards. During the experiment, 99 labeled seeds (1 seed for each of the 99 treatments) were randomly placed at each seed release point, resulting in a total of 891 artificial seeds per plot (99 seeds placed in each of the 9 seed release points). Each seed treatment was represented by 45 seeds spread evenly across the 5 plots (9 seeds per plot). In total, 4455 seeds (9 seeds each in 5 plots in each of the 99 seed treatments) were placed in the plots.
Investigation of seed fate

To record the number of seeds harvested by rodents we searched for tagged seeds around each plot after days 1, 2, 3, 4, 6, 8, 12, 16, 20, and 28. Based on our previous studies [12,18,23], rodents in this forest usually cache seeds less than 20 m from the harvesting site. We therefore, conducted a complete search within 20 m of each plot in all directions to relocate the removed seeds. We also conducted an extra search in a larger area in order to relocate as many of the seeds as possible. We made sure that we employed equal effort for each plot, i.e. two hours of three people for each visit. When we found a cache, we recorded the seed number, seed fate, and its exact location, including the directional angle and distance to the original seed source. We then reburied the seeds in order to minimize disturbance. We also surveyed the seed re-caching process (i.e. cached seeds were often repeatedly excavated and re-caught at another site by rodents). However, we focus only on the first seed movement in this study, as too few seeds were re-cached to conduct any meaningful analysis (only eight secondary caches and one tertiary cache were found). Based on our previous studies [9], seed fates were first divided into two categories (Fig. 1): (1) harvested by rodents and (2) ignored, i.e. left intact in situ. Harvested seeds were then determined to be either eaten (i.e. obviously gnawed by rodents or totally consumed with only plastic tags and a few seed fragments on the ground) in situ or removed by rodents; and removed seeds were determined to be cached (buried intact in the soil or deposited intact on the soil surface), eaten after being transported (removed by the rodents from the original release plots before being eaten), or missing (seeds that were not found within the search area).

Data analysis

We performed several analyses according to our different objectives, using the statistical programming language R (v. 3.1.0) [38]. Generalized linear mixed model (function glmer, package ‘lme4’) [39] was used to analyze seed fates with a binomial error distribution and logit link function. The dependent variables were bivariate variables and three models were built representing three distribution and logit link function. The dependent variables were 'lme4') [39] was used to analyze seed fates with a binomial error

Effects of Seed Size and Energy on Rodent Behavior

A linear Mixed-effects Model (function lmer, package ‘lme4’) [39] was used to analyze the effects on seed dispersal distance. We fitted the response variable (distance to seed release point) to a normal distribution after log-transformation. We used the same structure of random effects here as in the models above. Fixed effects were seed size (including both linear and quadratic terms) and energy content, and harvest time was added as a covariate. However, we couldn’t get p-values from function lmer, and we computed the p-values by the lmerTest package [40] based on Satterthwate’s approximations.

In all cases, to choose the best model and get the appropriate p values we did model simplification by using a likelihood ratio test and parsimony criteria. Thus, nonsignificant interactions and terms were removed to achieve the minimal adequate model.

Results

Seed harvest

Most of the artificial seeds (71.3%, n = 4455) were harvested, while 1279 seeds (28.7%) remained intact at the releasing plots at the end of the experiment. Energy content was a strong predictor of seed harvest (Table 1): seeds with higher energy content were harvested more frequently than seeds with less energy content (Fig. 2A). There was a significant quadratic term in seed size, while the linear term in seed size was not significant (Table 1): the negative sign of the terms meant that the medium-sized seeds were harvested more often than both large- and small-sized seeds (Fig. 2A). There was a significant interaction between the linear term in seed size and energy content (Table 1), and the positive coefficient showed that the slope between the probability of seed harvest and its energy content is larger for large-sized seeds. The negative interactive effect between the quadratic term in seed size and energy content indicated that the quadratic effect of seed size on seed harvest was much stronger for low-energy seeds (Fig. 2A, Fig. S2).

Seeds removal vs. in situ consumption

Of the 3176 seeds harvested by rodents, 46.3% were eaten in situ, while 53.7% were removed. Seed removal versus consumption in situ were highly affected by seed size, as revealed by the linear and quadratic terms in the model. Energy content alone had no significant effect (Table 1). Medium-sized seeds had the highest ratio of removal to in situ consumption by rodents.

Figure 1. Definitions of different seed fates during the scatter-hoarding process.
doi:10.1371/journal.pone.0111389.g001
followed by large- and small-sized seeds (Fig. 2B). The quadratic term in seed size and energy content also showed a significant interactive effect (Table 1): when seeds were of high energy content, rodents had a strong preference for medium-sized seeds; on the other hand, if seeds were of low energy content, rodents had a strong preference for medium-sized seeds; the interactive effect (Table 1): when seeds were of high energy content had no significant effect (Table S2). However, the absolute quantity of medium-sized seeds found was still larger than both large- and small-sized seeds (Table S2). Energy content showed no significant effect, while dispersal distance was found to be a strong predictor on whether a seed would be cached or eaten after being removed by rodents (Table 1). The farther a seed was dispersed, the more likely it was cached (Fig. 3). Harvest time also showed a highly significant effect (Table 1): seeds removed by rodents at the beginning of the experiment were removed to a farther distance than seeds harvested later in the experiment.

**Seeds cached vs. consumption after removal**

Of the 768 removed seeds found, most (76.3%) were eaten while only 182 seeds (23.7%) were found cached. Medium-sized seeds were cached rather than being directly consumed much more often than both large- and small-sized seeds (Table 1, Fig. 2D). Energy content showed no significant effect, while dispersal distance was found to be a strong predictor on whether a seed would be cached or eaten after being removed by rodents (Table 1). The farther a seed was dispersed, the more likely it was cached (Fig. 3). Harvest time also showed a highly significant effect (Table 1): seeds removed by rodents at the beginning of the experiment were removed more often than seeds removed later in the experiment.

**Discussion**

In this study, both seed size and energy content were significantly related to rodent foraging behavior, but their effects varied according to the different stages of the scatter-hoarding process. In general, rodents preferred medium-sized and high energy content seeds. High energy seeds were harvested at much

---

**Table 1. Summary of the generalized linear mixed models to test the variables affecting seed fate.**

| Fixed effects | Estimate ± SE | Z-value | P-value |
|---------------|---------------|---------|---------|
| Harvested vs. Ignored (Model I), n = 4455 | | | |
| Intercept | −4.171 ± 0.883 | −4.723 | <0.001 |
| Size | 0.279 ± 0.443 | 0.629 | 0.530 |
| Size Squared | −0.252 ± 0.113 | −2.224 | 0.026 |
| Energy | 8.860 ± 0.773 | 11.465 | <0.001 |
| Size x Energy | 7.426 ± 1.228 | 6.050 | <0.001 |
| Size Squared x Energy | −1.232 ± 0.311 | −3.964 | <0.001 |
| Removed vs. Eaten in situ (Model II), n = 3176 | | | |
| Intercept | −3.586 ± 0.562 | −6.383 | <0.001 |
| Size | 4.542 ± 0.575 | 7.901 | <0.001 |
| Size Squared | −0.834 ± 0.129 | −6.458 | <0.001 |
| Energy | −0.635 ± 0.778 | −0.815 | 0.415 |
| Day | 0.001 ± 0.007 | 0.110 | 0.912 |
| Size x Energy | 0.841 ± 0.885 | 0.950 | 0.342 |
| Size Squared x Energy | −0.398 ± 0.200 | −1.990 | 0.047 |
| Cached vs. Eaten after removed (Model III), n = 768 | | | |
| Intercept | −0.244 ± 1.239 | −0.197 | 0.844 |
| Size | 1.781 ± 0.994 | 1.792 | 0.073 |
| Energy | −0.938 ± 1.587 | −0.591 | 0.555 |
| Distance | 0.300 ± 0.146 | 2.052 | 0.040 |
| Size Squared | −0.365 ± 0.176 | −2.072 | 0.038 |
| Day | −0.199 ± 0.025 | −7.977 | <0.001 |
| Size x Energy | −0.981 ± 0.922 | −1.064 | 0.287 |
| Size x Distance | −0.160 ± 0.097 | −1.646 | 0.100 |
| Energy x Distance | −0.301 ± 0.206 | −1.461 | 0.144 |
| Size x Energy x Distance | 0.190 ± 0.134 | 1.416 | 0.157 |

The total number of individuals used (i.e. sample size) in each analysis are shown. doi:10.1371/journal.pone.0111389.t001
Figure 2. Comparison of fates for seeds with different sizes and energy content levels. There are 99 treatments of artificial seeds with 11 seed sizes x 9 levels of energy content. The sample size for each treatment is 45, i.e. 9 seeds x 5 plots.
doi:10.1371/journal.pone.0111389.g002

Table 2. Summary of the linear mixed-effects model to test the variables affecting seed dispersal distance (the sample size is $n = 768$).

| Fixed effects    | Estimate ± SE | t-value | P-value |
|------------------|---------------|---------|---------|
| Intercept        | 1.095 ± 0.311 | 3.518   | <0.001  |
| Size             | 0.954 ± 0.221 | 4.313   | <0.001  |
| Energy           | -0.532 ± 0.367| -1.449  | 0.148   |
| Size Squared     | -0.266 ± 0.042| -6.289  | <0.001  |
| Day              | -0.015 ± 0.006| -2.479  | 0.013   |
| Size x Energy    | 0.272 ± 0.170 | 1.595   | 0.111   |

doi:10.1371/journal.pone.0111389.t002
more rapid rates while seed size had a limited effect on harvest rate. On the other hand, seed size was more important for the remaining scatter-hoarding stages: seed removal or consumption in situ, decision on dispersal distance, and seed caching or consumption after removal.

As discussed in the introduction, seed size is usually estimated by seed mass and seed volume. In this study, we used seed volume (i.e., seed diameter) to indicate the size of our artificial seed as seed diameter is much more easy to artificially control, though most of the current studies used seed mass [4,5,19,29,31]. However, rodent foraging behavior responds similarly to seed mass and seed volume [22,23]. For example, Holl and Lulow found that neither seed length nor seed weight was correlated with the proportion of seed predation [22], while Wang et al. found both seed mass and seed length were positively correlated to the probability of seeds being harvested and cached [23]. Furthermore, seed mass and seed volume are usually positively related to each other and can both be measures of the energy content of a seed [10,12,19,23,24]. So, in the following discussion, we just used the word ‘seed size’.

Numerous studies have shown significantly positive relationships between seed size and scatter-hoarding rodent preferences for seed harvest, removal, caching and dispersal distance [4,5,19,29,31]. Our results indicated the existence of an optimal seed size, specifically medium-sized seeds, which maximized the energetic benefit to rodents while reducing handling costs. Similar results were also indicated by several other studies [9,30,35]. Optimal foraging models indicate animals maximize the net rate [34] or efficiency of foraging [41] with time as the primary limiting factor. Although larger seeds might contain more energy, manipulating larger seeds usually requires longer handling time [42]. Thus choosing the largest seed may not maximize the net rate of energy gain. Furthermore, increased handling time may also expose animals to greater predation risk [43]. The tradeoff between foraging benefit and predation risk may also prevent rodents from choosing the largest seeds. Pereira et al. also indicated that higher predation risk might decrease the seed handling time by wood mice, thus influencing their seed selection preference [44]. In this study, we have a much larger range of seed size in the artificial seed system than the natural species in the field. It may be argued that our finding that rodent prefer medium-sized seeds is an artifact which comes from that medium-sized artificial seeds are the maximum size a rodent in our study area can meet. Nevertheless, we should note that the observed preference for medium-sized seeds must be seen in relation to rodent body size, as the rodents we surveyed were quite small (only about 10 cm in length without the tail and tens of grams in weight). Rodent body size would mediate whether or not there is a seed size beyond which handling and transport become increasingly difficult and expensive [9,11]. However, the rodents in this study were indeed able to handle these large seeds although they were much larger than the maximum sized natural seeds. The appendix of the artificial seeds (i.e. the steel thread and plastic tag) might make the handling and transporting of this large seeds easier for the small rodents. Furthermore, we reanalyzed our data by only consider artificial seeds falling within the natural seed size limits (0.4–1.5 cm), and the results also showed a negative sign of quadratic term in seed size for seed harvest, removal and caching (Table S3), but with a weaker pattern than that with the whole range (Fig. S4); there was no significant negative sign of quadratic term in seed size for the dispersal distance (Table S4, Fig. S4).

Many studies have indicated that the energy content of seeds could influence seed harvest, seed removal, and also the dispersal distance [5,19,23,28,45]. Our results suggest that higher energy content rapidly increased seed harvest rate, but the extremely low energy content of our most energy poor artificial seeds may have been the driving force behind much of this observation. Energy content showed no significant effect on seed removal, seed caching and the dispersal distance, and this could be the consequence of rodents harvesting mainly the high energy seeds, hence, leaving no variation in seed energy content for the later stages of the process. However, there might be an alternative explanation, i.e. the overwhelming effects of seed size may attenuate the effect of energy content [12]. Seed size usually has a direct bearing on handling time, and thus foraging efficiency [34,41,42], especially for the small-bodied mouse species in our study. Kerley and Erasmus also found that rodents avoided high energy seeds which tended to be large and with long handling times [46].

Our results showed that dispersal distance influenced whether a seed would be cached or eaten after removal, with the result that seeds dispersed farther were more likely to be cached rather than eaten. Transporting seeds has a high energy cost, which may positively relate to dispersal distance, which may be particularly true for small-bodied rodents. It would be reasonable to assume that rodents should cache a seed after long distance transporting because of the energy investment already incurred for carrying the seed. However, this is not the case during the re-caching process because seed re-caching increases their dispersal distance but decreases the survival probability of the cached seed [7,47]. This might be because re-caching increases the number of seed-rodent encounters, thus increasing the probability of seed consumption [7,47]. While seed size, energy content and dispersal distance are important for caching, other potential factors may also play an important role on the foraging decision of caching vs. eating the seed, e.g., seed tannin content, destination microhabitat, seed germination schedule, etc. [7,16,21,48]. Furthermore, our study suggested that seeds harvested at the beginning of the experiment would be transported to a farther distance than seeds harvested later in the experiment, with a higher probability of being cached rather than eaten. This significantly negative effect of harvest time on dispersal distance and caching probability might be indirectly affected by seed traits. Rodents usually harvested their preferred seeds (e.g. seeds with more fat or energy) more rapidly [3,20,35], and this were also true in our case. Rodents harvested seeds with medium sizes or more energy content more rapidly (Fig. S5).

In our experiment, more than half the removed seeds (53.0%, n = 1706) were not found, thus, their fate is unknown. Seed size showed significant effects on whether a removed seed was found or not with the result that medium-sized seeds were found least often,
followed by large- and small-sized seeds (Table S2). Energy content showed no significant effect (Table S2). This difference in seed recovery rates would, if anything, lead to an underestimation of the proportion of seeds that were cached and the dispersal distance, particularly so for medium-sized seeds. Some studies indicated that these missing seeds might be dispersed beyond the search radius [4,49,50], which may be true in our case too. However, it is also possible that not all missing seeds were cached beyond the search radius. In our study area, seeds were usually found transported by rodents with less than 20 m, even when there was a much smaller proportion (e.g. 9.6% and 18.3%) of missing seeds [23,37]. The dominant rodents in our study site are usually small bodied, which might be a limiting factor for transporting seeds to far distances. Larder-hoarding behavior might be an alternative explanation for the missing seeds [51,52]. In our study, the line tied to the tags was about 15 cm, thus the tags might not be detected on the surface if rodents carried the seeds to deep burrows. This later presumption might be supported by the fact that medium-sized seeds were most frequently missed which is the preferred seed size by rodents.

Both seed size and energy content played important roles on the scatter-hoarding rodent foraging process, but varied according to the different stages. By using artificial seeds, we effectively teased apart the effect of seed size and energy content on the initial seed handling (i.e. seed harvest stage). In the later stages of the rodent foraging process, seed size played a critical role while energy content was of minimal importance. However, only relatively few low energy seeds were left after the seed harvest stage which may have biased the effects of energy in the later stages. Nevertheless, our findings shed light on scatter-hoarding rodent foraging behavior, and more specifically, demonstrate that there maybe important evolutionary tradeoffs imposed on seed size of rodent-dispersed species.

Supporting Information

**Figure S1** Artificial seeds made from clay and peanut powder. (JPG)

**Figure S2** Relations between seed size and seed harvest at different levels of energy content. (JPG)

References

1. Vander Wall SB (1990) Food Hoarding in Animals. Chicago: University Chicago Press.
2. Forget PM, Milleron T (1991) Evidence for secondary seed dispersal by rodents in Panama. Oecologia 87: 596-599.
3. Vander Wall SB (2001) The evolutionary ecology of nut dispersal. Botanical Review 67: 74-117.
4. Jansen PA, Bongers F, Hemerik L (2004) Seed mass and mast seedling enhancement dispersal by a neotropical scattering-hoarding rodent. Ecological Monographs 74: 569-589.
5. Xiao ZS, Wang VS, Harris M, Zhang ZB (2006) Spatial and temporal variation of seed predation and removal of sympatric large-seeded species in relation to innate seed traits in a subtropical forest, Southwest China. Forest Ecology and Management 222: 46-54.
6. Beck MJ, Vander Wall SB (2010) Seed dispersal by scatter-hoarding rodents in arid environments. Journal of Ecology 98: 1300-1309.
7. Perea R, San Miguel A, Gil L (2011) Acorn dispersal by rodents: the importance of re-dispersal and distance to shelter. Basic and Applied Ecology 12: 432-439.
8. James GA, PA, Hirsch BT, Ensens WJ, Zamora-Gutierrez V, Wiebke M, et al (2012) Thieving rodents as substitute dispersers of megafaunal seeds. Proceedings of the National Academy of Sciences of the United States of America 109: 12610-12615.
9. Wang B, Ye GX, Cannon CH, Chen J (2013) Dissecting the decision making process of scatter-hoarding rodents. Oikos 122: 1027-1034.
10. Vander Wall SB (2003) Effects of seed size of wind-dispersed pines (Pinus) on secondary seed dispersal and the caching behavior of rodents. Oikos 100: 23-34.
11. Munoz A, Bonal R (2008) Are you strong enough to carry that seed? Seed size/ body size ratios influence seed choices by rodents. Animal Behaviour 76: 709-715.
12. Wang B, Chen J (2009) Seed size, more than nutrient or tannin content, affects seed caching behavior of a common genus of Old World rodents. Ecology 90: 3023-3032.
13. Steele MA, Knowles T, Briddle K, Simms EL (1993) Tannins and partial consumption of acorns - implications for dispersal of oaks by seed predators. American Midland Naturalist 130: 229-238.
14. Steele MA, Gavel K, Bachman W (1998) Dispersal of half-eaten acorns by gray squirrels: effects of physical and chemical seed characteristics. In: Steele MA, Merritt JF, Zegers DA, editors. Ecology and evolutionary biology of tree squirrels. 223-231.
15. Kollmann J, Coomes DA, White SM (1998) Consistencies in post-dispersal seed predation of temperate fleshy-fruited species among seasons, years and sites. Functional Ecology 12: 691-699.
16. Smallwood PD, Steele MA, Faeth SH (2001) The ultimate basis of the caching preferences of rodents, and the oak-dispersal syndrome: tannins, insects, and seed germination. American Zoologist 41: 840-851.
17. Guinazuas PR, Jose J, Galenti M, Trigo JR (2003) Quinazolinone alkaloids in Ormosia arbores seeds inhibit predation but not hoarding by agoutis (Dasyprocta leporina). Journal of Chemical Ecology 29: 1065-1072.
18. Wang B, Chen J (2011) Scatter-hoarding rodents prefer slightly astringent food. PloS One 6: e26424.
19. Moore JE, McEuen AB, Swihart RK, Contreras TA, Steele MA (2007) Determinants of seed removal distance by scatter-hoarding rodents in deciduous forests. Ecology 88: 2529–2540.
20. Wang B, Chen J (2012) Effects of fat and protein levels on foraging preferences of tannin in scatter-hoarding rodents. Plos One 7: e49040.
21. Steele MA, Smallwood PD, Spanar A, Nelsen E (2001) The proximate basis of the oak dispersal syndrome: detection of seed dormancy by rodents. American Zoologist 41: 652–664.
22. Holt KD, Ludow ME (1997) Effects of species, habitat, and distance from edge on post-dispersal seed predation in a tropical rainforest. Biotropica 29: 459–468.
23. Wang B, Wang G, Chen J (2012) Scatter-hoarding rodents use different foraging strategies for seeds from different plant species. Plant Ecology 213: 1329–1336.
24. Perea R, Miguel AS, Martinez-Jaurripi M, Valbuena-Carabana M, Gil I (2012) Effects of seed quality and seed location on the removal of acorns and beech nuts. European Journal of Forest Research 131: 623–631.
25. Blake GM, Peart DR, 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.
26. Moles AT, Drake DR (1999) Potential contributions of the seed rain and seed bank to regeneration of native forest under plantation pine in New Zealand. New Zealand Journal of Botany 37: 83–93.
27. Moles AT, Warton DI, Westoby M (2003) Do small-seeded species have higher survival through seed predation than large-seeded species? Ecology 84: 3148–3161.
28. Vander Wall SB (1995) The effects of seed value on the caching behavior of yellow pine chipmunks. Oikos 74: 533–537.
29. Forget PM, Milleron T, Feer F (1998) Patterns in post-dispersal seed removal by neotropical rodents and seed fate in relation to seed size. In: Newbery DM, Prins HHT, Brown ND, editors. Dynamics of tropical communities. 25–49.
30. Theimer TC (2003) Intraspecific variation in seed size affects scatterhoarding behaviour of an Australian tropical rain-forest rodent. Journal of Tropical Ecology 19: 95–98.
31. Xiao ZS, Zhang ZB, Wang YS (2004) Dispersal and germination of big and small nuts of Pinus jeffreyi (Pinaceae): potential effects on plant reproductive success. Canadian Journal of Zoology 76: 154–162.
32. Xiao ZS, Zhang ZB, Wang YS (2008) Dispersal and germination of big and small nuts of Quercus serrata in a subtropical broad-leaved evergreen forest. Forest Ecology and Management 209: 187–195.
33. Smallwood PD, Peters WD (1986) Gray squirrel food preferences: the effects of tannin and fat concentration. Ecology 67: 168–174.
34. Stephens DW, Krebs JR (1986) Foraging Theory. Princeton University Press.
35. Jansen PA, Bartholomew M, Bongers F, Elzinga JA, Den Ouden J, et al. (2002) The role of seed size in dispersal by a scatter-hoarding rodent. In: Levey D, Silva WR, Galetti M, editors. Seed Dispersal and Frugivory: Ecology, Evolution and Conservation. 209–225.
36. Bartholomew EL (2001) The effects of tannin and protein on food preference in eastern grey squirrels. Ethology Ecology & Evolution 13: 115–132.
37. Wang B, Chen J (2008) Tannin concentration enhances seed caching by scatter-hoarding rodents: An experiment using artificial ‘seeds’. Acta Oecologica-International Journal of Ecology 34: 379–383.
38. R Development Core Team (2014) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available: http://www.R-project.org/
39. Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using S4 classes. R package version 1.1–7. http://cran.r-project.org/web/packages/lme4/index.html.
40. Kuznetsova A, Brockhoff PB, Christensen RHB (2014) lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package, version 2.0–6. http://cran.r-project.org/web/packages/lmerTest/index.html.
41. Waite IA, Ydenberg RC (1994) Shift towards efficiency maximizing by grey jays hoarding in winter. Animal Behaviour 48: 1466–1469.
42. Jacobs LF (1992) The effect of handling time on the decision to cache by grey squirrels. Animal Behaviour 45: 522–524.
43. Lima SL, Valone TJ, Caraco T (1985) Foraging-efficiency predation-risk trade-off in the grey squirrel. Animal Behaviour 33: 153–165.
44. Perea R, Gonzalez R, San Miguel A, Gil I (2011) Moonlight and shelter cause differential seed selection and removal by rodents. Animal Behaviour 82: 717–723.
45. Chang G, Jin TZ, Pri JF, Chen XN, Zhang B, et al. (2012) Seed dispersal of three sympatric oak species by forest rodents in the Qining Mountains, Central China. Plant Ecology 213: 1633–1642.
46. Kerley GIH, Erasmus T (1991) What do mice select for in seeds? Oecologia 86: 261–267.
47. Vander Wall SB, Joyner JW (1998) Recaching of Jeffrey pine (Pinus jeffreyi) seeds by yellow pine chipmunks (Tamias amoenus); potential effects on plant reproductive success. Canadian Journal of Zoology 76: 154–162.
48. Xiao Z, Zhang G, Zhang Z (2008) Testing the high-tannin hypothesis with scatter-hoarding rodents: experimental and field evidence. Animal Behaviour 75: 1253–1261.
49. Xiao ZS, Zhang ZB, Wang YS (2004) Dispersal and germination of big and small nuts of Quercus serrata in a subtropical broad-leaved evergreen forest. Forest Ecology and Management 195: 141–150.
50. Vander Wall SB (2002) Masting in animal-dispersed pines facilitates seed dispersal. Ecology 83: 3508–3516.
51. Jenkins SH, Beek SW (1998) Differences in food hoarding among six species of heteromyid rodents. Journal of Mammalogy 79: 1221–1233.
52. Li HJ, Zhang ZB (2005) Effects of mast seeding and rodent abundance on seed predation and dispersal by rodents in Prunus armeniaca (Rosaceae). Forest Ecology and Management 242: 511–517.