Factors influencing repeated seed movements by scatter-hoarding rodents in an alpine forest

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Scatter-hoarding rodents are effective dispersal agents for many plant species. Several studies have shown that rodents repeatedly re-cache seeds. The re-caching process often has a significant impact on final seedling establishment, but the factors determining its occurrence are poorly understood. In this study, we followed the fate of 3564 artificial seeds that varied in size, nutrient content and tannin content. Seeds cached closer to their original releasing plots were more likely to be re-cached, and to a further distance. Larger seeds were more likely to be re-cached than smaller ones, while nutrient and tannin content had little effect. Most plant species that depend on scatter-hoarding rodents for seed dispersal bear relatively large seeds, and large seeds are usually more likely to be dispersed and to establish seedlings, suggesting that the caching preferences of scatter-hoarding rodents may have played an important role in the evolution of large seeds.
seeds with univariate seed traits, we aimed to address the following predictions: 1) Seeds with larger size and higher nutrient or tannin content are more likely to be re-cached; 2) Seeds cached closer to their original releasing plots are more likely to be re-cached.

**Results**

During our live-trapping census, we obtained seventeen rodents from the night traps, but none from the day traps. Among the seventeen rodents, ten were *Apodemus chevrieri* (56.3%) and seven were *Apodemus latronum* (37.5%).

Most of the experimental seeds were harvested from the release points within the first three days after placement (96.9%, N = 3564). At the end of the experiment, we found 1333 primary caches, 209 secondary caches, 19 tertiary caches, and 2 quaternary caches (Fig. 1). On average, higher-order caches (i.e., 2nd and 3rd) were located further from the release points than were primary caches: seed size experiment- 13.6 ± 1.6 m (mean ± s.e.) and 12.3 ± 1.8 m vs. 6.9 ± 0.4 m (Linear mixed-effects model, t = 6.683, P < 0.001), nutrient content experiment- 19.5 ± 1.6 m and 34.4 ± 3.5 m vs. 10.3 ± 0.5 m (t = 10.184, P < 0.001), and tannin content experiment- 41.1 ± 1.0 m and 20.2 ± 3.9 m vs. 8.5 ± 0.3 m (t = 7.565, P < 0.001). Primary caching distance was positively related to seed size (Pearson's product-moment correlation, R² = 0.973, P < 0.001) and nutrient content (R² = 0.782, P = 0.004), while negatively related to both hydrolysable (R² = 0.865, P < 0.001) and condensed tannin content (R² = 0.824, P = 0.002)²⁴. Secondary caching distance (i.e. the distance from the release point to the secondary caches) was positively related to seed size (R² = 0.957, P < 0.001) and negatively related to hydrolysable tannin content (R² = 0.809, P = 0.002), but not significantly related to nutrient content (R² = 0.279, P = 0.179) or condensed tannin content (R² = 0.451, P = 0.099) (Fig. 2).

Seed dispersal distance increased significantly for successive movements. From primary to secondary caches it increased by 9.6 ± 1.5 m in the seed size experiment (Paired t-test, t₉₉ = 6.493, P < 0.0001, two-tailed), 9.6 ± 1.4 m in the nutrient experiment (t₉₈ = 6.986, P < 0.0001), and 8.1 ± 0.9 m in the tannin experiment (t₉₉ = 8.901, P < 0.0001). From secondary to tertiary caches it increased by 4.8 ± 1.3 m in the seed size experiment (t₇ = 3.556, P = 0.009), 17.0 ± 4.1 m in the nutrient content experiment (t₇ = 4.192, P = 0.013), and 10.0 ± 2.4 m in the tannin content experiment (t₇ = 4.204, P = 0.008) (Fig. 3).

The likelihood of seeds being excavated from primary caches and re-cached decreased with distance from the release point in the tannin content experiment (Generalized linear mixed model, Z = −3.131, P = 0.002), but not in the seed size experiment (Z = −0.117, P = 0.907) or the nutrient content experiment (Z = 1.039, P = 0.299) (Fig. 3). The likelihood of seeds being excavated from primary caches and re-cached increased with seed size (Z = 3.929, P < 0.001), but not with nutrient content (Z = 1.007, P = 0.314) or tannin content (Z = −0.931, P = 0.352). No interactions between primary cache distance and seed size, nutrient content or tannin content were found to affect the likelihood of primary cached seeds being re-cached (Z = −1.496, P = 0.145; Z = −1.052, P = 0.293; Z = 0.714, P = 0.475; respectively) (Table 1).

**Discussion**

The removal of seeds further from parent plants when re-caching brings a significant fitness advantage to seed dispersal²⁵. In this study, a large proportion of seeds from the primary caches were excavated by rodents and dispersed for a second time, reaching up to four successive dispersal movements. Seed dispersal distances increased significantly for successive movements, with the maximum distance up to 77.3 m.

Our tannin experiment indicated that current caching distance might be a major factor that influenced the re-caching process. Seeds with current caches closer to the original releasing plots were more likely to be re-cached, and to further distances. These results are consistent with the rapid-quuestering hypothesis.²⁶ Most of the experimental seeds (98.6%, n = 1620) were harvested from the release points within the first three days after placement and successive re-caching reduced the cache densities and presumably decreased the probability of pilferage. However, we did not record which animal made the original cache, so we could not tell if the re-caching was by the same individual. Cache pilferage by conspecific or heterospecific scatter-hoarders could give the same results, because pilferers are more likely to find and pilfer seeds that are closer, as higher seed density could improve foraging ability²⁷. Furthermore, the tagging method used in our study might encourage pilfering behavior by providing an obvious indicator of a cached seed and making it easy for naïve rodents to locate caches²⁸,²⁹; and this may make it less likely that many of these re-caching events involved recovery by the original cache owner.

However, current caching distance was found to have little effect on the re-caching process in both the seed size and nutrient experiments. In the nutrient experiment, seeds at primary caches, whether they were re-cached or not, were already dispersed relatively far away from the seed releasing plots (9.8 ± 1.1 m and 10.4 ± 0.5 m, respectively) (Fig. 3), perhaps reducing the likelihood of selective re-caching; while in the seed size experiment, the overwhelming effect of seed size on the re-caching process might attenuate the effects of distance; similar overwhelming effects of seed size were also found during the primary caching process when rodents initially encountered a seed³⁰.

In our study, seed size was positively related to re-caching probability and similar results were also found in some other studies³¹. Seed size usually directly indicates seed quality and may be much easier to assess than other seed traits (e.g. nutrient or tannin content). Furthermore, it is logical that a larger amount of cached food supply may be more likely to be relocated by rodents than a smaller one (either by smell or using visual clues), and/or may increase the motivation of potential thieves to locate and steal caches because of the greater reward per unit search time. Nutrient and tannin content of seeds had no effect on the re-caching process, although both factors significantly influenced the primary caching process at the rodents’ initial encounter with a seed³²–³⁴. After the initial foraging decision at the seed releasing plots, certain seeds were chosen to be scatter-cached by rodents based on several criteria, while others were either ignored or eaten in situ³⁵,³⁶; thus the variations in nutrient and tannin content among the cached seeds would be too limited to affect rodent re-caching decisions.

However, in the wild, nutrient quality (e.g. lipid reserves) and tannin content of seed are sometimes correlated with the germination delay, i.e. lipid and tannin-rich seeds sprouted later than those with less lipid and tannin, the former and later being used as long- and short-term term reserve for animals, respectively³⁷–³⁹. Independently of nutrient type, seed dormancy (especially in the temperate regions) might be also be an important factor, and it is well known in the literature that sprouting affects the chance of germinating seeds being re-cached, with the result that non-germinating seeds are more likely to be re-cached than germinated ones³⁹–⁴³. Some studies found that the germ can be excised (pruning) when re-caching occurs, which prevents germination and allows the conservation of the food supply by reducing the perishability of seed reserves during the establishment phase³⁷. Thus, finally, seed size, nutrient content, tannin content and timing of germination might be interacting factors that determine whether or not seeds are re-cached. However, it is impossible to test the timing of germination when using artificial seeds; furthermore, using peanut powder (lipid-rich) to make artificial seeds likely affected re-caching compared to using other materials, e.g. flour powder (sugar-rich). A very interesting comparison would be the fate of artificial seeds made out of two contrasting components (lipid vs. sugar), although it will still be impossible to monitor how factors such as rapid (for sugar-rich
Figure 1 | Diagram of seed fates, showing the percentages and numbers of seeds for each fate category during the re-caching process by scatter-hoarding rodents (EIS, eaten in situ; EAD, eaten after dispersed; LAP, left at plots; EAC, eaten at current caches; LAC, left at current caches). (a): seed size experiment: seeds with different size; (b): nutrient experiment: seeds with different nutrient content; (c): tannin experiment: seeds with different tannin content.
seeds) or delayed (lipid-rich) germination determine scatter-hoarding rodent decisions to re-cache primarily cached seeds. For that, it would be important to use wild seed species with different attributes, seed size and nutrient composition, and to monitor them during the establishment period during the fall through spring in future studies.

The level of competition may also be an important factor influencing the seed re-caching process, as it has been found to be positively related to the rates of cache pilferage\(^4\)-\(^6,\(^{21}\). However, we did not test the competition level as our study was conducted in one year across a small scale, when both food abundance and rodent populations were average. In our study, we checked seed fates 11 times after release, and the length of the census intervals increased during the study. It is possible that seeds were moved multiple times within an interval, and Jansen et al.\(^7\) even found that seeds were re-cached twice in a single day. This may be the reason that there were only few scattered occurrences of 3rd and 4th levels of re-caching in our study, because the repeated re-caching process was found to increase the probability of seed consumption by rodents\(^11\). Furthermore, more than half the seeds (56.2%, \(n = 3564\)) were missing with their fate unknown in our study, and were omitted from the analyses. Some of these missing seeds might have been dispersed beyond our search radius (\(\approx 20\) m). Hirsch et al.\(^{24}\) suggested that these incomplete data sets may bias the dispersal results, especially the seed dispersal kernel, because long-distance dispersal events were the least likely to be observed, and they also developed a 'censored tail reconstruction' method to provide unbiased estimates of long distance movements. However, it is still difficult to assess the repeated movements of missing seeds as they were not tracked.

In this study, we used artificial seeds to separate the effects of seed size, nutrients and tannin content on the seed re-caching process. The likelihood of seeds being re-cached increased with seed size, but not with nutrient content or tannin content. Furthermore, seed size also demonstrated a much more consistent and overwhelming effect than nutrient and tannin content on the primary foraging decision at the rodents’ initial encounter with a seed\(^25\). Most plant species that depend on scatter-hoarding rodents for seed dispersal bear relatively large seeds\(^8\)-\(^11,\(^{35-\text{5}6}\). Larger seeds usually have a much greater chance of being scatter-cached while small ones have a greater chance of being eaten\(^12\)-\(^17,\(^{34}\). Meanwhile, large seeds were often more likely to produce seedlings\(^26\). We therefore believe that the foraging preference for large seeds by scatter-hoarding rodents may have played an important role in the evolution of large seeds in these taxa\(^39\)-\(^41\).

**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: XTBG2007-004). We signed a contract (No. 20070017) with the Shangri-La Alpine Botanical Garden in 2007, and the contract included the permissions to access the study site and conduct this study.

**Study site.** The experiment was conducted during September to November in 2007, in a natural forest at the Shangri-La Alpine Botanical Garden (27° 54' N, 99° 38' E, altitude 3456 m), Yunnan province, southwestern China, where *Pinus densata* is the dominant tree species and the Sichuan field mouse (*Apodemus latronum*) and Chevrier’s field mouse (*Apodemus chevrieri*) are the two most abundant seed predators/dispersers\(^42\). Both rodent species have similar body sizes and foraging behaviour given the artificial seeds\(^42\).

**Experiment design.** The experimental data analyzed here was collected during September to November in 2007. The full description of the experiment design and the results of the primary seed fate from the release points have been already reported by Wang and Chen\(^25\). We used three univariate series of artificial seeds made from...
Analysis were conducted because of the limited sample size. Seed dispersal patterns were also found in the seeds from secondary caches (no statistical nor (b) nutrient content experiment (Generalized linear mixed model, caches and re-cached decreased with distance from the release point in (c) caching process.

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Figure 3 | Effects of current caching distance (mean ± s.e.) on seed re-caching process. The likelihood of seeds being excavated from primary caches and re-cached decreased with distance from the release point in (c) tannin content experiment (Generalized linear mixed model, \( Z = -3.131, P = 0.002 \)), but not in (a) seed size experiment (\( Z = -0.117, P = 0.907 \)) nor (b) nutrient content experiment (\( Z = 1.039, P = 0.299 \)); similar patterns were also found in the seeds from secondary caches (no statistical analysis were conducted because of the limited sample size). Seed dispersal distances increased significantly along re-caching processes (paired t-test, \( P < 0.05 \), two-tailed). Numbers besides the bars are the sample sizes.

clay, peanut powder and tannin to test the response of rodent foraging behaviour to three important seed traits: 1) Seed size - ten values: 0.2, 0.4, 0.6, 0.9, 1.2, 1.5, 2.0, 2.5, 3.0 and 4.0 cm in diameter (a total of 108 \( \times \) 10 = 1080 seeds), with each seed consisting of 50% peanut powder and 50% clay; 2) Nutrient content – eight values, with the percentage of peanut powder: 0, 10, 20, 30, 40, 50, 60 and 70 (totaling 108 \( \times \) 8 = 864 seeds), with each seed the same size (1.5 cm in diameter); 3) Tannin – seven

Six plots (2 m \( \times \) 2 m) > 50 m apart were set up in the forest to conduct the three univariate-trait experiments separately. At each plot, we located 9 subplots in 3 \( \times \) 3 grids, with about 1 m between subplots. Each circular subplot was about 15 cm in diameter and the seeds were placed along the circle with the tags located outwards. Two seeds of each value of size (or nutrient or tannin content) were placed on each subplot. In total, 108 seeds of each kind were spread evenly over the six plots (2 seeds \( \times \) 9 subplots \( \times \) 6 plots). We checked the plots and recorded seed harvest (i.e. seeds eaten in situ or removed away from their releasing point) 11 times, i.e. the 1\(^{\text{st}}, 2\^{\text{nd}}, 3\^{\text{rd}}, 4\^{\text{th}}, 6\^{\text{th}}, 8\^{\text{th}}, 10\^{\text{th}}\), 12\^{\text{th}}, 16\^{\text{th}}, 20\^{\text{th}}, 28\^{\text{th}} and 36\^{\text{th}} day after seed placement. Rodents in this forest were usually found to move seeds less than 20 m based on our previous studies\(^{12,25}\), so we 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. When we found a cache, we recorded whether the cached seeds were intact or eaten, and the exact location with a directional angle and the distance to their original seed sources. At the end of each visit, we checked all caches identified in the previous visit, until they were removed or eaten by rodents. If a marked cache was removed, then the area around the cache (radius of at least 20 m) was searched. Seed fates were grouped into the following categories: 1) left at plot, leaving intact at the original release plot; 2) eaten in situ/eaten at caches, leaving only plastic tags and seed fragments on the ground surface of the original release plot/current caches; 3) cached, buried intact in the soil or deposited intact on the soil surface; 4) eaten after dispersed, removed by the rodents from the original release plots/current caches before being eaten, 5) left at current caches, leaving intact at current caches until the end of the experiments; 6) missing, seeds that were not found within the search area, hence with an unknown fate.

Survey for rodents’ community. During the same period of seed placement, live-traps were baited with fresh peanuts to detect the key rodent species that were affecting seed predation/dispersal. In order to minimize the trapping effect on the rodent population in the plots where the artificial ‘seeds’ were released, the trapping plots were about 500 m away but in the same forest. Five transects were selected and five trap stations at an interval of 10 m were set along each of the five transects for six consecutive days and nights. At each trap station, two living traps were set together with their orientation reversed. Traps were checked every day at 7:30 am and 6:00 pm, and the numbers of captured rodents were recorded.

Data analysis. We performed several models according to different objectives, and plot was treated as a random effect. Generalized linear mixed model (package ‘lme4’) was used to analyze the effects of current dispersal distance and seed traits on seed fate, i.e. re-cached or not. Linear mixed-effects model (package ‘nlme’) was used to analyze the effects of seed trait and cache order on dispersal distance. Pearson’s product-moment correlation was used to test for significance of the relationships affecting seed predation/dispersal. In order to minimize the trapping effect on the rodent population in the plots where the artificial ‘seeds’ were released, the trapping plots were about 500 m away but in the same forest. Five transects were selected and five trap stations at an interval of 10 m were set along each of the five transects for six consecutive days and nights. At each trap station, two living traps were set together with their orientation reversed. Traps were checked every day at 7:30 am and 6:00 pm, and the numbers of captured rodents were recorded.

Table 1 | Summary of the generalized linear mixed model to test the effects of current dispersal distance and seed traits on seed fate (i.e. re-cached or not)

| Seed size Experiment | Estimate ± SE | Z-value | P-value |
|----------------------|---------------|---------|---------|
| Size                 | 1.336 ± 0.340 | 3.929   | 0.000   |
| Distance             | -0.012 ± 0.102| -0.117  | 0.907   |
| Nutrient content     | -0.073 ± 0.049| -1.496  | 0.135   |
| Nutrient × Distance  | 1.308 ± 1.300 | 1.007   | 0.314   |
| Nutrient content     | 0.048 ± 0.047 | 1.039   | 0.299   |
| Nutrient × Distance  | -0.122 ± 0.116| -1.052  | 0.293   |
| Tannin               | -1.847 ± 1.983| -0.931  | 0.352   |
| Distance             | -0.082 ± 0.026| -3.131  | 0.002   |
| Tannin × Distance    | 0.185 ± 0.259 | 0.714   | 0.475   |

Figure 3 | Effects of current caching distance (mean ± s.e.) on seed re-caching process. The likelihood of seeds being excavated from primary caches and re-cached decreased with distance from the release point in (c) tannin content experiment (Generalized linear mixed model, \( Z = -3.131, P = 0.002 \)), but not in (a) seed size experiment (\( Z = -0.117, P = 0.907 \)) nor (b) nutrient content experiment (\( Z = 1.039, P = 0.299 \)); similar patterns were also found in the seeds from secondary caches (no statistical analysis were conducted because of the limited sample size). Seed dispersal distances increased significantly along re-caching processes (paired t-test, \( P < 0.05 \), two-tailed). Numbers besides the bars are the sample sizes.

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