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PLANT-MICROBE-ANIMAL INTERACTIONS - ORIGINAL RESEARCH

Tree-to-tree variation in seed size and its consequences for seed dispersal versus predation by rodents

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Abstract Individual variation in seed size and seed production is high in many plant species. How does this variation affect seed-dispersing animals and, in turn, the fitness of individual plants? In this study, we first surveyed intraspecific variation in seed mass and production in a population of a Chinese white pine, *Pinus armandii*. For 134 target trees investigated in 2012, there was very high variation in seed size, with mean seed mass varying among trees almost tenfold, from 0.038 to 0.361 g. Furthermore, 30 of the 134 trees produced seeds 2 years later, and for these individuals there was a correlation in seed mass of 0.59 between years, implying consistent differences among individuals. For a subset of 67 trees, we monitored the foraging preferences of scatter-hoarding rodents on a total of 15,301 seeds: 8380 were ignored, 3184 were eaten in situ, 2651 were eaten after being cached, and 395 were successfully dispersed (cached and left intact). At the scale of individual seeds, seed mass affected almost every decision that rodents made to eat, remove, and cache individual seeds. At the level of individual trees, larger seeds had increased probabilities of both predation and successful dispersal: the effects of mean seed size on costs (predation) and benefits (caching) balanced out. Thus, despite seed size affecting rodent decisions, variation among trees in dispersal success associated with mean seed size was small once seeds were harvested. This might explain, at least in part, the maintenance of high variation in mean seed mass among tree individuals.

Keywords Cache · Individual variation · Scatter hoarding · Seed production · Species interaction

Introduction

Individual variation in seed size and number is high in many plant species (Thompson 1984; Wulff 1986; Sork et al. 1993; Jacquemyn et al. 2001; Herrera and Jovani 2010). Many studies have focused on the potential trade-off between seed size and the number that a tree can produce, and the possible ecological and evolutionary consequences of this trade-off (Smith and Fretwell 1974; Venable 1992; Jakobsson and Eriksson 2000; Kosinski 2010). Other studies have investigated the causes of variation in seed production (Parciak 2002; Zas and Sampedro 2015) and the consequences of this variation for seed germination and for seedling growth and survival (Stanton 1984; Susko and Lovett-Doust 2000; Gomez 2004). Although seed dispersal and predation by animals are critical processes for recruitment for many plant species (Janzen 1971; Howe and Smallwood 1982; Herrera 2002; Vander Wall 2010), the question of how variation in seed size and number at the individual plant scale affects seed dispersal and dispersal agents has received relatively little attention (Herrera et al. 1994; Jordano 1995; Martinez et al. 2007; Shimada et al. 2015).

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For plants with animal-dispersed seeds, the probability of seed dispersal and survival depends on the foraging preferences of seed dispersers, which in turn likely depends on characteristics of the seeds or fruits themselves (Jordano 2000; Vander Wall 2010; Lichti et al. 2015). For example, scatter-hoarding rodents are important seed dispersers for many tree species, but whether they act as seed dispersers or seed predators may depend on both seed size and number of seeds available (Jansen et al. 2004; Vander Wall 2010; Xiao et al. 2013, 2015; Lichti et al. 2015). There is growing evidence that scatter-hoarding rodents prefer to disperse and cache larger seeds, but eat in situ or ignore smaller seeds, both for seeds from a single species and seeds from multiple species (Vander Wall 2003, 2010; Jansen et al. 2004; Xiao et al. 2006a; Wang et al. 2012; Lichti et al. 2015). Furthermore, of those seeds that are dispersed, larger seeds are usually dispersed farther (Jansen et al. 2004; Moore et al. 2007; Wang and Chen 2009; Vander Wall 2010; Wang et al. 2014). The number of seeds available can also affect the foraging behaviour of scatter-hoarding rodents. For example, rodents can show a reduced probability of dispersing seeds or a decreased dispersal distance in years with high seed production compared with seed-poor years (Jansen et al. 2004; Theimer 2005; Klinger and Rejmanek 2010; Xiao et al. 2013; but for differing results, see Vander Wall 2002; Yi et al. 2011).

Current studies focusing on how seed size and seed density affect foraging behaviour of scatter-hoarding rodents mainly target one of three scales of comparison: (i) the among-species scale, with comparisons of dispersal and predation of seeds from multiple co-occurring species (Vander Wall 2003; Moore et al. 2007; Wang et al. 2012); (ii) the among-population scale, with comparisons among stands or years (Jansen et al. 2004; Xiao et al. 2005); or (iii) the individual seed scale, with comparisons among individual seeds from the same species (Jansen et al. 2004; Xiao et al. 2013; Shimada et al. 2015). Even though individual variation in reproductive success within populations is a key component of evolutionary fitness (Clark et al. 2001; Herrera and Jovani 2010; Moran and Clark 2012), variation in seed dispersal and predation at the scale of individual trees is poorly understood. By releasing individually weighed seeds to mimic seed size and number from individual trees of oil tea (a rodent-dispersed species), Xiao et al. (2015) tested whether seed size (individual seed scale) and number (individual tree scale) affected seed dispersal, and showed that seed dispersal increased with seed size and decreased with seed number at the source trees. However, because differences in mean seed size among individual trees were not assessed, this study did not allow a full investigation of the effects of seed size and number on the dispersal-related fitness of tree individuals. Shimada et al. (2015) showed that within-tree variation of seed mass could result in differences in seed removal by rodents among trees, and they discussed this question from the perspective of foraging patch selection by animals. However, because the total seed production and final seed fate were not assessed, this study could not separate seed dispersal from predation by scatter-hoarding rodents. To our knowledge, Xiao et al. (2015) and Shimada et al. (2015) are the only two studies that have addressed rodent–plant interactions at the scale of individual trees.

Our study asks how variation in seed mass and number among tree individuals affects the behaviour of animal dispersers and in turn the component of tree fitness involving dispersal of seeds away from the mother tree. This was motivated by the very large range in mean seed mass (roughly an order of magnitude) that we found among individual trees of our study species, Chinese white pine (*Pinus armandii*). We first surveyed intraspecific variation in seed production in a natural population in south-western China. We then investigated how this variation affects seed predation and dispersal by scatter-hoarding rodents, which were the primary seed dispersers/predators. In 2012, we weighed and then followed the fate of 15,301 seeds from 67 tree individuals to determine their survival and, if they survived, the distance they were dispersed. In 2013, a year with no seed production at our study site, we used 2010 seeds obtained from an outside commercial source to conduct a similar experiment. In this experiment, we placed the commercial seeds under selected trees to mimic the seed distribution that would have occurred if trees had produced seeds. This makes it possible to contrast 2012 with high natural seed production to 2013 with low seed production. Furthermore, we used commercial seeds, which factored out any differences in the size and quality of seeds among mother trees; therefore, the analyses reveal the specific effects of seed size on rodent foraging and dispersal success independent of tree-to-tree variation in seed size and/or quality. We analysed the data to test whether there were trade-offs in the effects of seed mass on successful seed dispersal versus seed predation. This allowed us to address whether the behaviour of scatter-hoarding rodents could help to explain the high variation in mean seed mass among tree individuals.

Materials and methods

Study area and study species

Our study was conducted in a pine forest in the Hengduanshan Mountains in Shangri-La county, Yunnan Province (28°00′N, 99°32′E, alt. 2830 m). The mean annual temperature is 5.4 °C, and annual rainfall is 625 mm. The forest is mostly primary growth, dominated by Chinese white pine and Sikang pine (*Pinus densata*). The understory is dominated by shrubs in the genera *Quercus* and *Rhododendron*. The ground flora is poorly developed, with only a few herbs and mosses. Chinese white pine is an evergreen species native to China which produces large wingless seeds in September and October. Scatter-hoarding rodents and nutcrackers (*Nucifraga caryocatactes*) are the primary seed dispersers and predators across the tree species' range (Chen and Chen 2011; Wang et al. 2012). Chen and Chen (2011) describe a spatial mosaic in which rodents or nutcrackers are regionally the more important dispersers, and at our study site dispersal and predation by nutcrackers were seldom observed. In our live-trap census, the Sichuan field mouse (*Apodemus latronum*) and Chevrier's field mouse (*A. chevrieri*) were the two most abundant seed dispersers/predators. Both species have similar body size and show similar foraging behaviour (Wang and Chen 2011).

Seed collection

A 70 × 160 m plot was established in the forest for the field study. In September 2012, all the Chinese white pine trees that produced cones in the plot were labelled ($n = 134$), and their girths at breast height (GBH) were measured. All the cones from each labelled tree were collected with a long-arm tree pruner and left outdoors until they dehisced naturally, after which all the seeds from each cone were collected and weighed singly. The time between removal and dehiscence was roughly 10 days. Because nutcrackers remove seeds before cones dehisce, our collection of cones close to dehiscence ensured that any observed removal of seeds from cones before we collected them gives a good representation of the impact of nutcrackers on seed predation. Shrivelled seeds were excluded during seed collection, and no seed was found to be infested by insects. We planned to conduct the seed collection for three consecutive years; however, no seeds were collected in 2013 because few trees produced cones in the forest, and none in our study plot. In 2014, thirty of the labelled trees produced cones, and all the cones were collected and then treated at the same way as in 2012. In 2012, approximately 165 (out of 52,083) seeds were removed by nutcrackers before cone collection; to correct for this, we estimated the number of seeds lost from the proportion of area of the cone pecked by nutcrackers and added these to the total. In 2014, a few cones naturally dehisced before we collected them, but no seed removal by nutcrackers was observed. We accounted for this loss of 359 seeds by counting the traces of seeds in the cones. Overall, the low incidence of seed removal by nutcrackers indicates low abundance of nutcrackers at the study site.

Seed dispersal experiment

In October, 2012, we selected 67 of the 134 labelled trees to represent the full range of seed mass and number observed; subsetting was necessary to make the scale of the seed dispersal experiment manageable. For each tree selected, all seeds were weighed and tagged using a modification of the methods of Xiao et al. (2006b). A 0.6-mm diameter hole was drilled at the base of each seed, and an individually numbered 2.5 × 0.7 cm red plastic tag (0.1 g) was tied through the hole with thin steel thread (15 cm in length). When rodents cached the seed in the soil, the tags were often left on the surface, making the seeds easy to locate. After tagging, seeds were returned to the ground around their own mother trees. Seed release points were set up randomly beneath the crown of each tree, with about 60 cm between points. The number of release points for each tree was equal to the number of cones it produced, and seeds from the same cone were released at the same point. At each release point, seeds were placed in a circle with the tags pointing outwards, with the diameter of the circle reflecting the number of seeds released (15–30 cm). We checked the tagged seeds at each release point to investigate the seed fates on days 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 74, 141, 234 and 365 after seed release. Tags may potentially facilitate cache recover by naive rodents if they learn the location of caches from the tags. However, our study targeted the effects of seed size on rodent foraging decisions during scatter hoarding, and all seeds were tagged regardless of size. Furthermore, in this study system tags show little effect on whether a seed is eaten or dispersed after detection by rodents (Wang et al. 2012), although this is not the case in other systems (Yi et al. 2008; Wrobel and Zwolak 2013). Xiao et al. (2006b) found that tags had no effects on cache survival.

In October, 2013, we bought Chinese white pine seeds from the Seed and Seedling Company of Yunnan to conduct the seed dispersal experiment. Because the commercial seeds varied less in mass than the seeds produced at our study site, we divided them into three size categories: large (0.32–0.34 g), medium (0.24–0.26 g), and small (0.16–0.18 g). One seed release point was set up at each of the 67 trees used in 2012, and 30 seeds (10 seeds in each size category) were released at each point in a circle with the tags pointing outwards. Because all the seeds were harvested (i.e. eaten in situ or removed) by rodents in the first 6 days, we checked seed fates on days 1, 2, 3, 4, 6, 8, 10 and 24 after release.

During both seed dispersal experiments (2012 and 2013), we completely searched an area of a radius of 25 m around each seed release point, and we further searched haphazardly beyond this area to relocate as many seeds as possible. When we found a cache, we recorded the seed fate and its distance and direction from the original release point. At subsequent visits, we continued to check all the caches found in the previous surveys until the cached seeds were eaten or removed again by rodents. If cached seeds were removed, then the area around the cache (radius of at least 25 m) was searched.

This procedure allowed us to assign seeds to categories based on the sequence of events (Wang et al. 2013; Wang and Yang 2014). Starting at their initial experimental release site, seeds were categorized as (i) ignored (left intact in situ) versus harvested; for those harvested, seeds were categorized as (ii) eaten in situ (i.e. leaving only plastic tags and seed fragments) or removed from their initial location; for those removed, seeds were categorized as (iii) eaten, (iv) missing, or (v) cached (buried intact in the soil or deposited intact on the soil surface). We then repeated this categorization scheme for those seeds that were cached, and repeated it again for those seeds that were cached a second time. Thus, these data give a detailed characterization of the fate of seeds, following seeds that were cached or re-cached up to three times. During the experiments, we found very few seeds (1.5 and 3.4% in 2 years, respectively) to be cached in situ (i.e. cached at their original seed release point), and these were also considered as being cached. Only a small proportion of cached seeds were buried in soil (2.3 and 1.4% in 2012 and 2013, respectively), while the majority of seeds were deposited intact on the soil surface. However, most of the seeds were covered with some litter.

Data analysis

The data on seed fate consisted of discrete outcomes (e.g. seeds were ignored vs. harvested, and seeds were cached vs. either eaten or missing). We used two different statistical approaches for these data: logistic regression with a Firth correction and a logistic generalized linear mixed model (GLMM) to account for variation due to source tree and cones that were included as random effects in the models. We used both approaches, because arguments could be made for and against incorporating random effects for trees and cones. Differences in the fates of seeds could depend on trees or cones due to factors other than seed size; for example, rodent activity could be greater under one tree than another, leading to greater chances of a seed being harvested. In this case, tree and cone should be included as random effects. The GLMM also allowed us to include a random effect for variation among trees in the relationship between the fates of seeds and their mass. The argument for using logistic regression without random effects is that there are large differences in mean seed mass among trees; in some of the analyses >80% of the variation in seed mass was explained by variation among trees and cones. When tree and cones are treated as random effects in these analyses, the random effects could potentially absorb most of the variation in the fate of seeds caused by variation in seed mass. Thus, we performed analyses with and without treating trees and cones as random effects.

Results

Variation in seed mass and seed number among trees

In 2012, 703 cones were produced by the 134 target trees, half of which produced only one or two cones. The 703 cones contained 52,083 seeds, and the mean seed crop per tree was 389 with a range from 19 to 2823 (Fig. S1a). The mean seed mass per tree varied from 0.038 to 0.361 g, with a mean of 0.153 ± 0.064 g (Fig. S2a, c). In 2014, 30 of the 134 target trees produced 81 cones and 4233 seeds, and the mean seed production per tree was 141 with a range from 5 to 475 (Fig. S1b). The per capita mean seed mass varied among tree individuals from 0.078 to 0.407 g, with a mean of 0.211 ± 0.085 g (Fig. S2b, d), which was significantly larger than that in 2012 (linear mixed model, $t = 39.4$, $P < 0.001$). For the 30 trees that produced seeds in both years, there was a positive correlation in per capita mean seed mass between years, implying consistent differences among individuals ($r = 0.590$, $P < 0.001$, Fig. 1a). However, the per capita number of seeds produced was not consistent between years ($r = 0.247$, $P = 0.186$, Fig. 1b).

Relationship between mean seed mass and seed number

We analysed the data by regressing log seed number per tree against square-root mean seed mass (to homogenize variances) and GBH. In both years separately (Table S1: 2012, $P = 0.003$; 2014, $P = 0.013$) and when combined ($P = 0.003$), there was a hump-shaped relationship between log seed number and square-root mean seed mass (Fig. 2). This suggests that high seed mass leads to lower seed number, although low seed number also occurs for trees with low seed mass. Note that the ratio (standard error)/(mean seed mass) per tree was on average 0.028, implying that the measurement error was small and did not affect the patterns in Fig. 2. Mean seed mass (logistic regression: $P = 0.11$) and seed number per tree ($P = 0.08$) in 2012 did not affect the probability that a tree produced seeds in 2014.

Effects of individual seed mass on seed dispersal by rodents in 2012

In 2012, 15,301 seeds of the 67 selected trees were released back under their mother trees. By the end of the

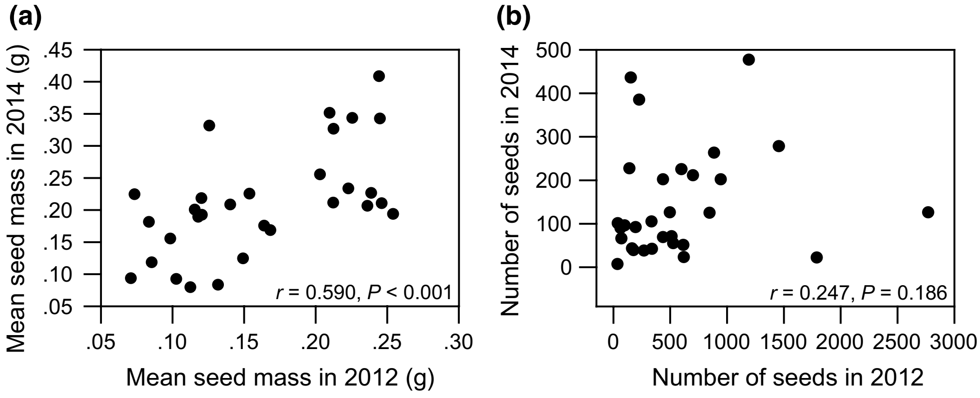

Fig. 1 Seed production per individual tree between years versus **a** mean seed mass and **b** seed number per tree, with Spearman rank correlations

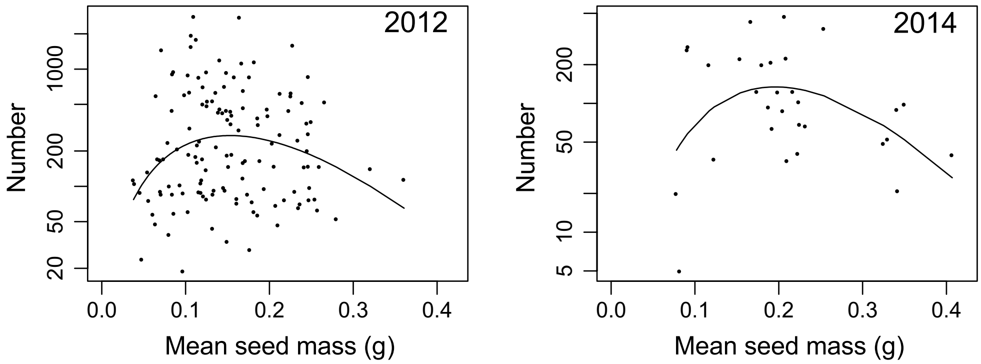

Fig. 2 Log number of seeds produced per tree versus mean seed mass (square-root transformed) in 2012 and 2014. *Fitted lines* are from quadratic regressions, and the second-order term is statistically significant for both years (Table S1)

experiment, nearly half of the released seeds ($n = 6921$) were harvested by rodents. Larger seeds were more likely to be harvested and, of those harvested, larger seeds were more likely to be cached (Fig. 3a; Tables S2, S3). By the end of the sequence of removing, eating and caching, larger seeds were more likely to be successfully dispersed (cached and then left intact) than smaller seeds (Table 1). The relationships between seed fates and seed mass were almost all the same whether the analyses were performed with or without random effects (Tables S2, S3).

At all rodent decision points until the seeds were removed from primary caches, the GLMM also identified tree-to-tree and cone-to-cone variation in the fate of seeds that was not explained solely by seed mass (σ^2[tree] and σ^2[cone] differed significantly from zero; Table S3). Furthermore, the effect of seed mass on seed fate differed significantly among trees until the seeds were removed from primary caches (σ^2[Seed Mass|tree] differed significantly from zero). To illustrate these tree-to-tree differences, Fig. 3 plots the relationships between seed mass and the probability of being harvested (Fig. 3b) and the probability of being removed (not eaten in situ) after harvesting (Fig. 3c) for each tree separately. These panels show the random effect of tree σ^2[tree] as the differences among trees in the probability of seeds of the same mass being harvested and removed: the slope random effect (σ^2[Seed Mass|tree]) appears as differences among trees in the slope with respect to seed mass within trees.

Of those seeds that were removed and then cached by rodents, larger seeds were carried farther than smaller seeds to the primary cache location (Fig. 3d). In contrast, for the seeds moved to a secondary cache, there was only a marginally statistically significant correlation between seed mass and dispersal distance from the original release point (Fig. 3e). Furthermore, being re-cached moved the seeds a greater distance from their original release point; the distances from secondary caches to the release point (4.54 ± 4.10, $n = 92$) were significantly farther than the primary caches (2.15 ± 2.46, $n = 2249$) (linear mixed model, $t = 10.22$, $P < 0.001$).

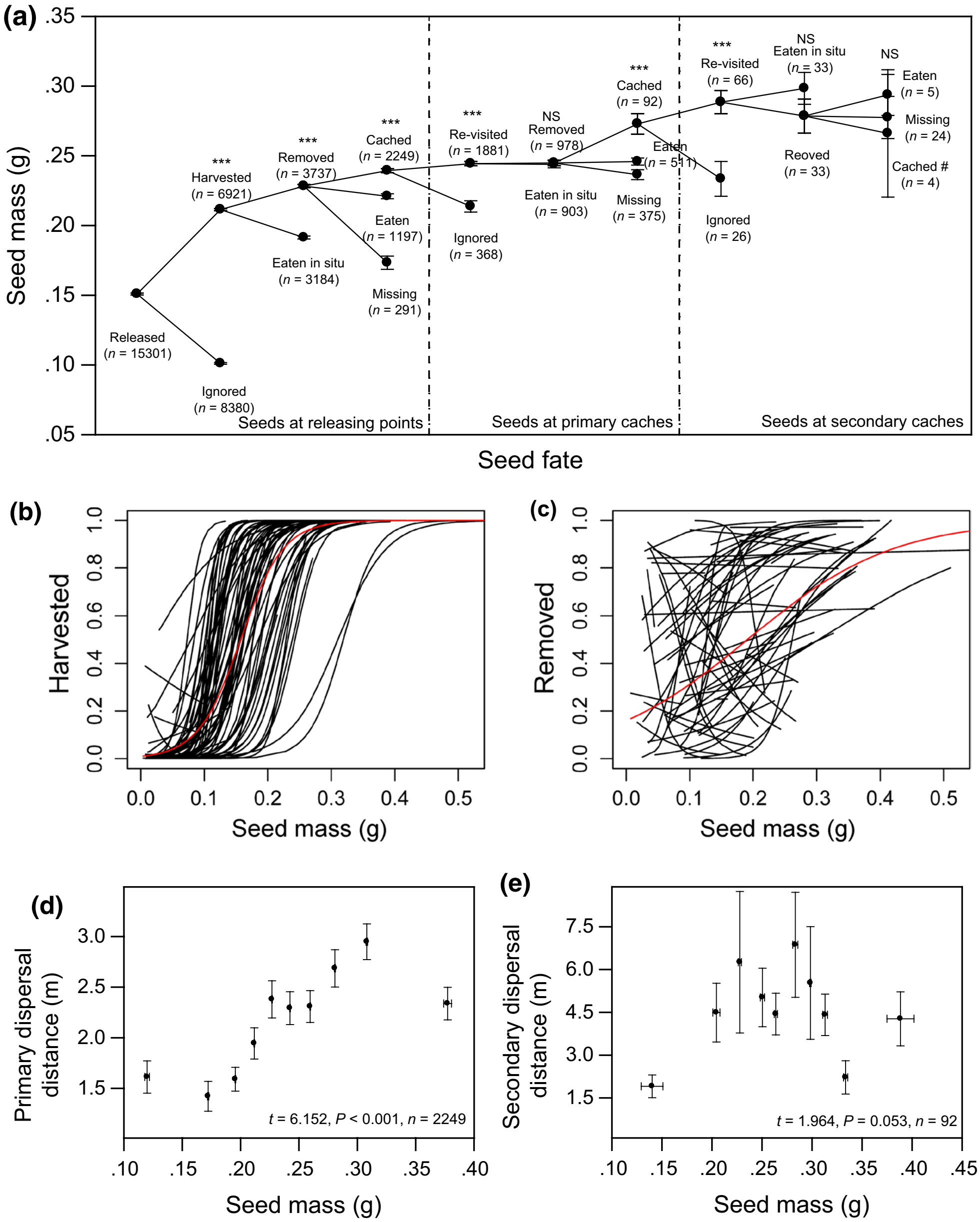

Fig. 3 Summary of the seed dispersal experiments in 2012 (combined data of the 67 sampled trees). **a** Seed fates after being released at their mother tree. Results comparing the differences of seed mass between seed fates (***$P < 0.001$; **$P < 0.01$) are presented for logistic regression with a Firth correction (Table S2), and the results for a GLMM were essentially the same (Table S3); missing seeds were not included during analysis because of their uncertain fates. For the 4 tertiary cached seeds (noted with #), two were eaten in situ and the other two were either missing or ignored. **b** Logistic regressions for each tree giving the probability that a seed is harvested (rather than ignored) under the mother tree versus seed mass. There is a separate line for each tree, and the *red line* shows the result for all seeds regardless of mother tree. Although the probability of harvesting increases with seed mass for all trees, differences in the *horizontal* locations of the lines show that, for a given seed mass, the probability of harvesting is much higher for some trees than others. **c** Logistic regressions for each tree giving the probability that a seed once harvested is removed (rather than eaten) versus seed mass. The separate lines for each tree show that the effect of seed mass on removal differs among tree individuals. **d** Relationship between dispersal distance of primary cached seeds and seed mass. Linear mixed models were used to test the effects of seed mass on dispersal distance at the seed individual level, with random effects for trees and cones. **e** Relationship between dispersal distance of secondary cached seeds and seed mass (color figure online)

Table 1 Generalized linear mixed model for the effects of seed mass on dispersal success (cached and then left) in 2012 and 2013

Year	Variable	Estimate ± SE	*P* value
2012			
$N = 15{,}301$	Intercept	−6.11	
	Seed mass	9.70 ± 0.96	<0.001
	σ^2[tree]	0.67	
	σ^2[cone]	0.93	
2013			
$N = 2010$	Small seeds	−3.14	
	M–S seeds	0.95 ± 0.20	<0.001
	B–S seeds	1.26 ± 0.19	<0.001
	σ^2[tree]	1.20	
	σ^2[cone]	0	
2012 and 2013			
$N = 3995$	Small seeds	−4.14	
	M–S seeds	0.79 ± 0.17	<0.001
	B–S seeds	1.20 ± 0.17	<0.001
	Year 2013	0.96 ± 0.32	0.003
	σ^2[tree]	1.46	
	σ^2[cone]	0.22	

In 2013, seeds were grouped into large (B, 0.32–0.34 g), medium (M, 0.24–0.26 g) and small (S, 0.16–0.18 g) categories, and for the comparison between 2012 and 2013, only those seeds within the same size categories in 2012 were included in the model

Effects of individual seed mass on seed dispersal by rodents in 2013

Lack of seed production in our study plot in 2013 gave the opportunity to investigate the effect of seed mass while factoring out any possible differences in seeds among mother trees. We simulated seed production by placing commercial seeds under surrogate mother trees, using the same procedure as in 2012 using seeds produced by mother trees. As in 2012, in 2013 larger seeds were more likely to be initially removed, while smaller seeds were more likely to be eaten in situ (Fig. 4a; Tables S4, S5). For seeds at primary caches (Fig. 4a), larger seeds were more likely to be ignored, although of those seeds re-visited, larger seeds were more likely to be removed and either re-cached or eaten. Beyond this point, the only statistically significant effect was that larger seeds were more likely to be removed from secondary caches (Fig. 4a; Tables S4, S5). Overall, larger seeds were more likely to be successfully dispersed than smaller seeds (Table 1). Furthermore, larger seeds were dispersed a greater distance than medium and small seeds at both primary (3.70 ± 3.94 vs. 2.42 ± 2.09 and 1.73 ± 1.72 m, mean ± SD) and secondary caches (10.04 ± 8.07 vs. 5.85 ± 4.12 and 3.78 ± 2.80 m) (Fig. 4b, c). As in 2012, there were significant random effects of tree (location) on the fate of seeds, but except for the first rodent decision point (removing seeds from the release point or not), the tree identity (location) did not affect the relationship between seed fate and seed size (Table S5).

To ask whether the effects of seed size were similar between 2012 and 2013, we had to include both data sets in the same statistical analysis. Although we recognize that the sources of seeds (natural vs. commercial) differ, we were interested in whether the same sized seeds were handled by rodents differently between years. Differences in total numbers of seeds dispersed between years would indicate a possible effect of differences in total seed production between years. Also, similar responses of rodents to seed size between years would indicate that seed mass, rather than differences among mother trees in mean seed mass or other seed qualities, is sufficient to explain rodent decisions during scatter-hoarding processes. To match the data sets as closely as possible, we categorized the naturally occurring seeds in 2012 into large, medium, and small to match the size categories in 2013. There was a statistically significant effect of year, with seeds in 2013 more likely to be successfully dispersed than seeds in 2012 (Table 1), but the interaction between year and seed size was not statistically significant (likelihood ratio test, $\chi^2_2 = 2.6$, $P = 0.27$). We repeated this analysis placing all seeds in 2012 into 3 size categories (rather than the narrower size categories of 2013), with equal numbers of seeds in each category, and the results were qualitatively the same (results not reported). Therefore, seed size had the same statistical effect on dispersal success in both years, even though the proportion of seeds that were harvested differed between years.

Two additional issues arise when comparing 2012 and 2013, because in 2013 we surveyed seeds for only 24 days, as compared to the entire year in 2012. First, almost all seeds were harvested (eaten or removed) in 2013, while only around half of the seeds in 2012 were harvested. Therefore, even though our survey efforts were less in 2013, we did not underestimate the proportion of seeds that were harvested. Second, secondary and tertiary seed predation might have been underestimated in 2013, as the survey was terminated earlier than in 2012. While this might have increased our estimates of seed dispersal success in 2013, there was no interaction between year and seed size, so that the effect of seed size was the same in both years.

Variation in seed dispersal among tree individuals

The previous analyses show that the size of individual seeds affects their fates, with larger seeds more likely to be removed and successfully dispersed. Because mean seed mass differed so greatly among trees, we investigated whether variation in mean seed size translates into differences in individual tree dispersal success. At the end of the

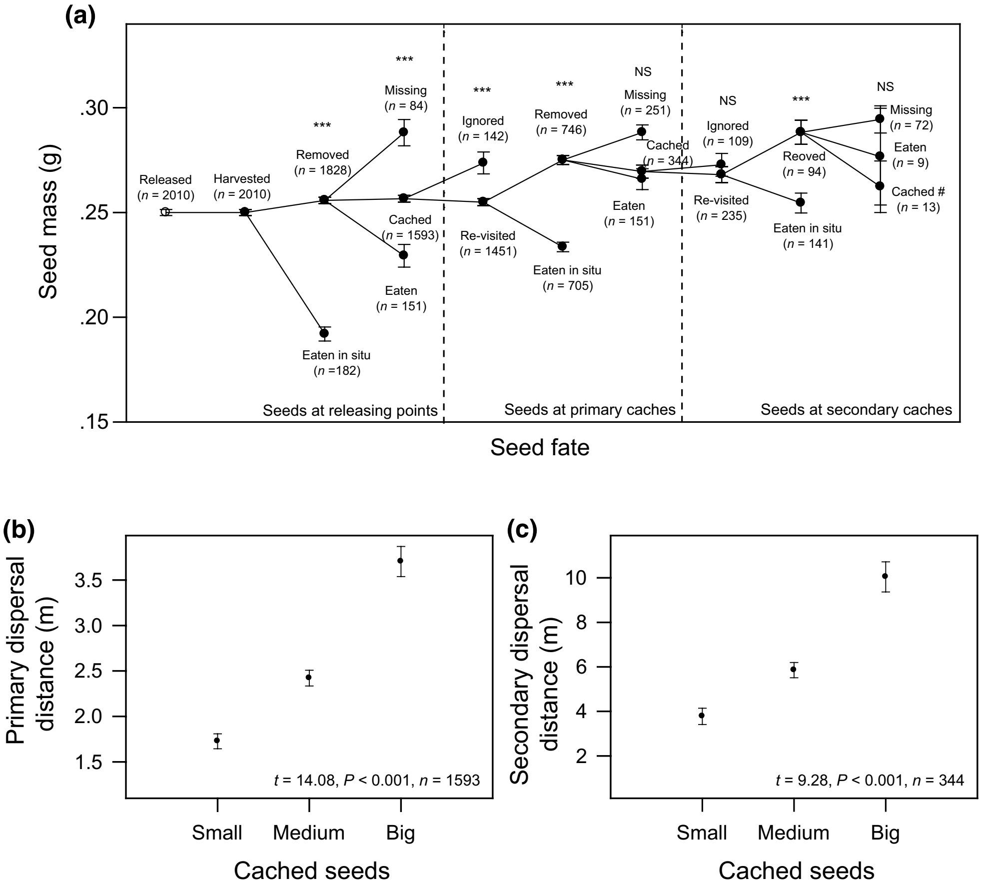

Fig. 4 Summary of the seed dispersal experiments in 2013. **a** Seed fates after being released at the 67 trees. Results comparing the differences of seed mass between seed fates (***$P < 0.001$; **$P < 0.01$) are presented for logistic regression with a Firth correction (Table S4), and the results for a GLMM were essentially the same (Table S5); missing seeds were not included during analysis because of their uncertain fates. For the 13 tertiary cached seeds (noted by #), four were eaten in situ, four were missing and the left five seeds were ignored. **b** Relationship between dispersal distance of primary cached seeds and seed mass. **c** Relationship between dispersal distance of secondary cached seeds and seed mass. In the models, small (0.16–0.18 g), medium (0.24–0.26 g) and large (0.32–0.34 g) seeds were assumed to have a mass of 0.17, 0.25, and 0.33 g, respectively

1-year experiment in 2012, 395 of the 15,301 released seeds were successfully dispersed (i.e. left in place after being removed and cached by rodents) (Fig. 3a). Large variation in successful seed dispersal existed among the 67 trees, with a range of 0–62 seeds successfully dispersed; this represented a range of 0–36% of successful seed dispersal. Trees that produced larger seeds had a greater proportion of their seeds successfully dispersed (Table 2; Fig. 5a). However, trees that produced larger seeds were also more likely to have their seeds eaten by rodents, either in situ or after being dispersed (Table 2; Fig. 5b). These two effects of seed size cancelled each other out; considering only those seeds that were harvested (i.e. either successfully dispersed or eaten, excluding those that were ignored at the initial release site), there was no effect of either seed size or number on the proportion dispersed (Table 2; Fig. 5c). Finally, at the level of individual trees, the effect of seed size on the mean ultimate dispersal distance of successfully dispersed seeds was not significant ($P = 0.071$, Table 2).

Discussion

Our survey found that mean seed mass ranged from 0.038 to 0.361 g among individual trees, and mean seed mass per tree was correlated between 2012 and 2014. While variation in mean seed size among individual trees from the same species is common (Thompson 1984; Wulff 1986; Jacquemyn et al. 2001; Gomez 2004; Shimada et al. 2015), we could find only one example in the literature of variation as high as that we found for Chinese white pine, the valley oak (*Quercus lobata*) reported by Koenig et al. (2009). What could explain the maintenance of such high variation in mean seed mass, given the importance of seed mass for

Table 2 At the level of individual trees, effects of mean seed mass and seed number on successful seed dispersal and seed eaten (excluding missing seeds from the analyses)

Fixed effects	Estimate ± SE	*t* value	*P* value
Proportion of seeds successfully dispersed			
Intercept	0.36 ± 0.75	0.48	0.63
Seed mass	**7.34 ± 2.83**	**2.59**	**0.012**
Seed number ($\times 10^{-6}$)	1.3 ± 294	3.42	0.98
GBH[†]	−0.006 ± 0.009	−0.66	0.51
Proportion of seeds eaten (both in situ and after being dispersed)			
Intercept	−2.49 ± 0.39	−6.38	<0.001
Seed mass	**13.00 ± 1.93**	**6.73**	**<0.001**
Seed number ($\times 10^{-6}$)	**302 ± 139**	**2.17**	**0.034**
GBH	−0.004 ± 0.005	−0.76	0.45
Proportion of seeds successfully dispersed relative to seeds eaten			
Intercept	−2.52 ± 0.75	−3.36	0.001
Seed mass	1.01 ± 3.49	0.29	0.77
Seed number ($\times 10^{-6}$)	−212 ± 278	−0.76	0.50
GBH	−0.003 ± 0.009	−0.37	0.71
Log dispersal distance of successfully dispersed seeds			
Intercept	0.31 ± 0.34	0.90	0.37
Seed mass	3.01 ± 1.62	1.85	0.071
Seed number	0.0003 ± 0.0002	1.11	0.27
GBH	−0.008 ± 0.005	−1.52	0.14

[†] Girth at breast height

seed and seedling success, and hence fitness (Wolfe 1995; Gomez 2004; House et al. 2010; Zas and Sampedro 2015)?

We found that the mass of individual seeds affected almost every step in their discovery, consumption, and dispersal by scatter-hording rodents (Figs. 3, 4). Rodents were more likely to harvest larger seeds under trees, were more likely to remove them rather than eat them in situ, and were more likely to cache and re-cache them. At the individual tree level, while large mean seed mass increased the proportion of seeds harvested by rodents, once harvested the advantages of large seeds via dispersal were counterbalanced by the disadvantages via seed predation, so that the mean seed mass conferred no advantage for successful dispersal (Fig. 5c). Thus, although successful dispersal away from the mother tree is an important component of fitness (Janzen 1971; Howe and Smallwood 1982; Levin et al. 2003), mean seed mass may have little effect on dispersal fitness of the mother tree: every decision that rodents make depends on seed mass, yet still the sum of these decisions (once seeds are found and harvested under the mother tree) does not lead to clear optimal seed mass that would drive stabilizing selection. Thus, the high variation in mean seed mass among trees could be maintained because mean seed mass is not under strong selection from rodent dispersers. This argument, however, requires several considerations and caveats.

We found that, after initial harvesting, seed mass does not increase the chances of successful dispersal. In 2013 when regional seed production by Chinese white pine was very low, and we used commercial seeds for experiments, all seeds were harvested by rodents. Therefore, in years of low seed production (or high rodent abundance), we would expect no effect of seed mass on dispersal success. In a seed masting year like 2012, however, only 45% (6921/15,301) of the seeds were harvested, and those left under the mother trees were relatively small. Therefore, larger seed mass could increase the overall success of seed dispersal if the success of ignored seeds was less than that for successfully dispersed seeds, 5.7% (395/6921). Unfortunately, we have no measure of seed and seedling success to compare between dispersed and ignored seeds, although we suspect that the success of ignored seeds under their mother tree is low (Janzen 1970; Connell 1971; Gross 1984; Jakobsson and Eriksson 2000). Furthermore, our field observations also showed that there were no seedlings under the tree canopies ($n = 134$). This implies a positive effect of mean seed mass on dispersal success, suggesting directional selection for larger seeds.

Balancing this possible positive effect of larger seeds through dispersal, we found some evidence for negative effects of larger seeds through reductions in seed number. Specifically, we found a hump-shaped relationship, with trees having high mean seed mass producing fewer seeds than trees with intermediate mean seed mass (Fig. 2). This might put an upper selective bound on mean seed size, limiting the positive directional selection through dispersal. Interpreting this hump-shaped relationship, however, requires caution, because even though trees showed consistent variation in mean seed mass between 2012 and 2014, we have no direct evidence that mean seed mass is heritable. We suspect that the hump-shaped relationship is caused by the interaction of genetic (Wolfe 1995; House et al. 2010; Zas and Sampedro 2015) and environmental (Roach and Wulff 1987; Parciak 2002) effects. Some trees are located in relatively poor conditions, leading to both low seed mass and small numbers, while the trade-off between mean seed mass and seed number is only seen for those trees located in good conditions (Sgro and Hoffmann 2004). We do not know whether any trade-offs might be persistent; however, since even though we found a consistent hierarchy among individuals in seed size between 2012 and 2014, this was not the case for seed number (Fig. 1).

Many studies on rodent-dispersed plants have shown that larger seeds are favoured during seed dispersal (Jansen et al. 2004; Moore et al. 2007; Vander Wall 2010). Previous studies on Chinese white pine also found that larger seeds had

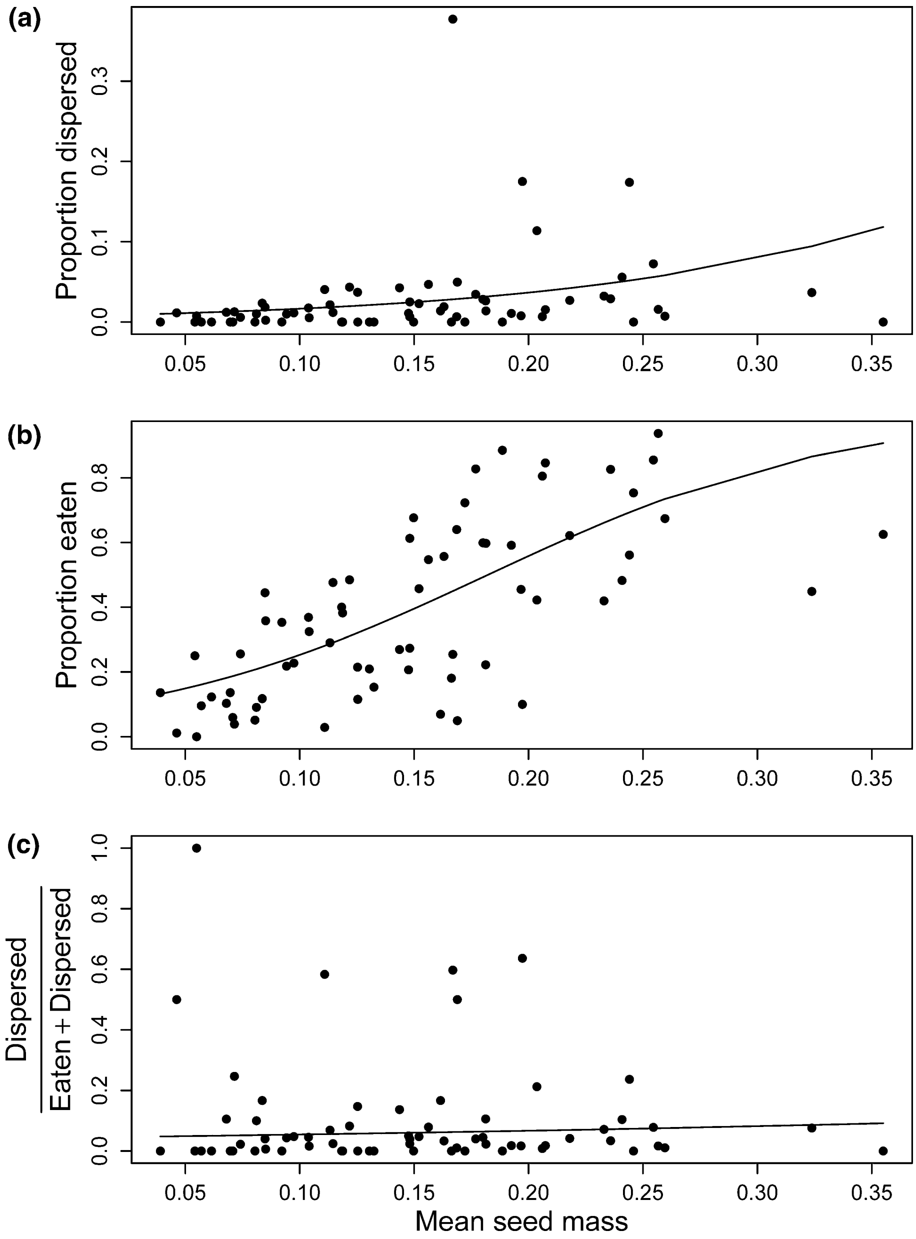

Fig. 5 At the level of individual trees, effects of mean seed mass on **a** the proportion of seeds successfully dispersed, **b** the proportion of seeds eaten (both in situ and after being dispersed), and **c** of those seeds harvested from the initial release site, the proportion successfully dispersed (see Table 2)

greater dispersal success than smaller ones (Chen and Chen 2011; Wang et al. 2012). However, to our knowledge no previous study has been able to investigate the effects of mean seed mass at the scale of individual trees on seed dispersal success by rodents. Although it might seem intuitive that the results from measuring the effects of individual seed mass on rodent behaviour will automatically predict dispersal success measured at the scale of individual trees, this is not necessarily the case and was not the case in our study. Even though at most decision points following harvesting rodents preferred to cache rather than eat larger seeds (Figs. 3a, 4a), this did not lead to trees with higher mean seed mass having greater dispersal success (Fig. 5c). The likely cause for this is that the effects of mass for seeds from different trees on rodent behaviour varied substantially (Fig. 3b, c). This variation could be explained, for example, if some trees (i) were closer to rodents' burrows, (ii) had greater brush underneath to provide rodents with cover, (iii) produced seeds with different chemical compositions, or (iv) dropped cones before or after the majority of other tree individuals (Perea et al. 2011; Takahashi et al. 2011). Of these, (i) and (ii) are supported by our experiment in 2013 in which commercial seeds were placed under surrogate mother trees. Analysis of these data showed variation among surrogate mothers in the effect of seed mass (Table 1), despite each tree having the same distribution of seed sizes. Furthermore, rodents might base their foraging decisions not on absolute seed mass but instead on relative seed mass (Shimada et al.

2015). This might explain why the probability of a rodent harvesting a medium-sized seed from a tree with low mean seed mass is greater than that from a tree with high mean seed mass (Fig. 3b, c). Given the many possible sources of the observed tree-to-tree variation in the effect of seed mass on rodent foraging, data on the dispersal success of seeds measured at the scale of individual trees were the only way to infer the possible fitness consequences of mean seed mass for tree recruitment success.

In addition to tree-to-tree variation in the effects of seed mass, we also investigated rodent predation and dispersal behaviour between 2 years: 2012 when seed production was high, and 2013 when it was low and we used commercial seeds for experiments. Although a much higher proportion of seeds was harvested in 2013 (essentially all, vs. 45% in 2012), we found little difference in the effects of seed mass between years either at the level of individual seeds (Tables S2–S5; Figs. 3, 4) or individual trees (Table 1). This is similar to other studies showing that scatter-hoarding rodents usually have consistent behaviours in response to seed size, regardless of the variation in both seed abundance and rodent population fluctuation among years (Vander Wall 2002; Jansen et al. 2004; Moore et al. 2007; Wang et al. 2012).

In this study, we showed that seed mass affects almost every decision made by scatter-hoarding rodents when harvesting, eating, and caching seed. Nonetheless, at the scale of individual trees, mean seed mass had no effect on overall seed dispersal success once seeds had been discovered and harvested underneath the mother tree. This leads to a possible explanation for the very high variation (0.038–0.361 g) in mean seed mass among trees. Our result also emphasizes that, in order to investigate the potential fitness consequences of seed size for dispersal, it is necessary to look at the scale of individual trees.

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Author contribution statement BW conceived, designed and performed the experiments. BW and ARI analysed the data and wrote the manuscript.

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