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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

High regeneration capacity helps tropical seeds to counter rodent predation

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Abstract Rapid germination of non-dormant seeds is one adaptation plants have evolved to counter seed predation by rodents. Some rodent species have evolved behaviors that prevent or slow the seed germination process through seed embryo removal or seed pruning; however, no plant species is known to have successfully escaped embryo removal or seed pruning by rodents. Here, we report that the non-dormant seeds of *Pittosporopsis kerrii* Craib in tropical rain forests in China have a high regeneration capacity to counter seed pruning by rodents. We found seed pruning, instead of embryo removal, was commonly used by rodents to increase food storage time by slowing down the seed germination process, but that *P. kerrii* seeds have a high regeneration capacity to escape seed predation by rodents: all pruned seeds, pruned roots and embryo-removed seeds by rodents or people retain the ability to develop into seedlings. Seeds of *P. kerrii* also have other capacities (i.e. rapid seed decomposition and indigestible dormant taproots) to escape predation by reducing the plant's attractiveness to rodents. The association between seed pruning behavior in rodents and high regeneration capacity of pruned seeds or roots in *P. kerrii* seeds are likely novel adaptation strategies adopted by seeds and rodents, respectively.

Keywords Embryo removal · Hoarding behavior · Seed dispersal · Seed germination · Seed pruning

Introduction

The seed–rodent system is an ideal model for studying the mutualism–predation association and coexistence between plants and animals in forests (Zhang et al. 2005). Rodents not only eat seeds but also disperse them and thus facilitate the seed regeneration of forests (Hulme 1997; Vander Wall 1990). Seeds and rodents have formed a very complex mutualism and predation relationship. For survival and regeneration, seeds have evolved one of two strategies: resistance or tolerance. Seed predation by animals leads to strong selection pressure on seed traits which in turn results in a variety of resistance-related traits in seeds (e.g., thick, spiny fruits, hard seed coats, or toxic chemical compounds) (Grubb et al. 1998; Janzen 1969; Rosenthal and Bell 1979; Vander Wall 2010; Zhang and Zhang 2008). However, high-level resistance (e.g., hard seed coat) often reduces the speed of seed removal by rodents and seed dispersal (Zhang and Zhang 2008). Tolerance traits in plant seeds can be an alternative mechanism for seed survival (Dalling and Harms 1999; Mack 1998; Vallejo-Marin et al. 2006; Xiao et al. 2007). This strategy favors seed dispersal and the formation of mutualism between seeds and rodents. Unfortunately, only

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a few studies of tolerance traits have been reported in the literature (Mendoza and Dirzo 2009; Xiao et al. 2007).

Germinated seeds can escape seed predation by rodents when their energy reserves in cotyledons or endosperms are converted into indigestible taproots or seedlings (Barnett 1977; Fox 1982; Hadj-Chikh et al. 1996; Jansen et al. 2006; Steele et al. 2001, 2006; Xiao et al. 2010). Thus, early seed germination of non-dormant seeds is likely an evolutionary adaptation to escape seed predation by rodents (Chang et al. 2009; Hadj-Chikh et al. 1996). However, some rodent species have evolved special behavioral strategies to prevent or delay seed germination during the hoarding process (Elliott 1978; Jansen et al. 2006; Xiao et al. 2009). Several squirrel species are able to arrest seed germination by removing the embryos of white oak acorns—embryo-removed acorns can be preserved for 6 months or more (Steele 2008). Eastern chipmunks (*Tamias striatus*) and red acouchies (*Myoprocta exilis*) use similar strategies to extend the storage time of non-dormant seeds of *Fagus grandifolia* and *Carapa procera*, respectively (Elliott 1978; Jansen et al. 2006).

Seed embryos are vital for seedling development, but are also very vulnerable to damage inflicted by rodents and other animals. Thus, embryo removal by rodents imposes strong selection pressure on trees and may have resulted in the evolution of seed traits to counter animal behavior that results in embryo excision (Steele 2008). However, to our knowledge, there are no studies of plant species that have successfully evolved strategies to counter embryo removal by rodents.

In this study, we investigated seed fates of intact seeds, pruned roots and pruned seeds of *P. kerrii* by rodents in the Menglun Nature Reserve, Xishuangbanna tropical forests, Yunnan Province, China. *P. kerrii* Craib is a dominant shrub species in the tropical rainforest of southern China (Lan et al. 2008). The seeds of *P. kerrii* germinate very quickly after falling to the ground, usually within about 1 week. Small rodents were commonly seen to prune the seminal roots of germinated seeds of *P. kerrii*. At the same time, both the pruned seeds and the pruned roots were seen to be established as normal seedlings. This study aims to test the following two hypotheses: (1) seed pruning may be used by rodents to increase storage time by delaying the seed germination process of the non-dormant seeds of *P. kerrii*, and (2) a high regeneration capacity of severed seeds may be an important strategy for *P. kerrii* to escape seed predation by rodents.

Materials and methods

Study site

The study was conducted in a tropical forest of the Menglun Nature Reserve, Xishuangbanna, Yunnan Province, China. We selected three stands for presenting and tracking tagged seeds of *Pittosporopsis kerrii* Craib from August 2007 to September 2009. Stand 1 was a tropical montane evergreen broad-leaved forest, while stand 2 was a tropical seasonal rain forest. Both stands are located near a permanent plot of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences (21°50′N, 101°12′E; elevation 780 m). Stand 3 was a secondary forest (30 years after prohibition of cultivation) located within the Xishuangbanna Tropical Botanical Garden (21°56′N, 101°15′E; elevation 550 m).

P. kerrii is the dominant shrub species in the tropical rainforest of Xishuangbanna region. It is also commonly seen in the tropical montane evergreen broad-leaved forest. Seeds of *P. kerrii* mature in August. The seed mass is 5.58 ± 1.40 g; length is 2.34 ± 0.22 cm; the largest diameter is 2.18 ± 0.20 cm; and the thickness of the seed coat is 0.72 ± 0.18 mm ($n = 98$). The seed has a large endosperm, a large dicotyledon, and a long radicle. Seeds germinate very quickly after falling to the ground, usually within about 1 week. The nutritional component of germinated seeds gradually transformed into a dormant taproot, which is indigestible to rodents. The seed body then soon separates from the taproot and immediately decomposes. Taproots then develop into seedlings within several months, depending on conditions. In the study sites, *Niviventer confucianus* is the dominant rodent species, though other rodent species (*Niviventer fulvescens*, *Rattus flavipectus* and *Maxomys surifer*) are commonly seen. Several tree squirrel species (*Dremomys rufigenis*, *Callosciurus erythraeus* and *Tamiops swinhoei*) were observed in the forest, but they are not highly abundant.

Seed releasing experiments

We selected three stands in the study area for releasing and tracking plastic-tagged seeds of *P. kerrii* Craib from August 2007 to September 2009. In August 2007 and 2008, 3,600 seeds were released in the three stands (540 seeds in stand 1 and 2 for each year, and 900 and 540 seeds, respectively, in 2007 and 2008 in stand 3). In each study stand, we placed 18 or 30 seed stations spaced about 10–20 m apart along a single transect. At each station, 30 tagged seeds were placed on the ground surface. The tagged seeds at each seed station were protected by using a steel wire-mesh enclosure (enclosure size: 0.7 × 0.7 × 0.5 m; mesh size: 1 × 1 cm), with one small hole (10 × 10 cm) on each side of the four walls to allow access by small rodents but prevent the entrance of large vertebrates. Seeds were marked by attaching a small coded plastic tag to each seed through a thin steel thread (Xiao et al. 2006; Zhang and Wang 2001). The ultimate fates (eaten, missing, dead, germinated or seedling establishment) of released seeds were surveyed at weeks 2, 4, 8, 12, 16 and 20 of the current year, and then in March, July and September of the following year.

Because fates of missing seeds were unknown, we concentrated our analysis on relocated seeds. The relocated seeds were classified into two types: intact seeds and pruned seeds. The pruned seeds were seeds that, based on the presence of pruning scars, we determined had experienced pruning of seminal roots by rodents. Seed pruning only occurred after seed germination (i.e. emergence of the seminal roots), and pruned seeds could re-sprout. Intact seeds were seeds that did not have evidence of root pruning during the survey process. The relocated seeds were also classified according to whether they were removed or not removed from the seed station. The ultimate fates of seeds were classified into three categories: consumed seeds, seedling establishment, and seed death. Consumed seeds referred to seeds that were eaten by rodents, seedling establishment referred to seeds that successfully developed into seedlings, and seed death referred to seeds which died naturally due to non-rodent predation (e.g., physiological or biological or environmental damage).

Seedling establishment of intact seeds, pruned seeds and pruned roots at and away from seed stations are illustrated in Fig. 1. Seedlings that developed from the pruned roots might be from relocated or missing seeds (all possible pruned seeds; Fig. 1). Thus, we defined the probability of seedling establishment of pruned seeds and pruned roots as the proportion of the total number of seedlings that emerged from pruned seeds and pruned roots to the total number of all pruned seeds. We defined the probability of seedling establishment of intact seeds as the proportion of the total number of seedlings that emerged from intact seeds to the total number of intact seeds.

Let us assume that:

1. The proportion of pruned seeds in missing seeds is same as that in re-located seeds;
2. Seeds are only pruned once, or pruned seeds produce only one pruned root;
3. Pruned seeds are not returned to the seed station after their initial removal.

For non-removed seeds at seed stations, let the number of missing seeds be M_1, the intact seeds be N_1, the pruned seeds be x_1, the pruned seeds removed from the seed stations be y, the seedlings that emerged from pruned roots be S_1, the seedlings that emerged from pruned seeds be T_1, the seedlings that emerged from intact seeds be H_1, p_i^1 is the probability of seedling establishment of intact seeds, and p_c^1 is the total probability of seedling establishment of pruned seeds and pruned roots:

$$p_i^1 = H_1/N_1 \tag{1}$$

$$p_c^1 = \frac{T_1 + S_1}{x_1 + M_1(x_1 + y)/(x_1 + N_1 + y)} \tag{2}$$

For removed seeds dispersed from the seed stations, let the number of missing seeds be M_2, the intact seeds be N_2, the pruned seeds be x_2, the seedlings that emerged from pruned roots be S_2, the seedlings that emerged from pruned seeds be T_2, the seedlings that emerged from intact seeds be H_2, p_i^2 is the probability of seedling establishment of intact seeds, and p_c^2 is the total probability of seedling establishment of pruned seeds and pruned roots:

$$p_i^2 = H_2/N_2 \tag{3}$$

$$p_c^2 = \frac{T_2 + S_2}{x_2 + M_2(x_2 - y)/(x_2 + N_2 - y)} \tag{4}$$

For all relocated seeds, let the number of missing seeds be M_0, the intact seeds be N_0, the pruned seeds be x_0, the seedlings that emerged from pruned roots be S_0, the seedlings that emerged from pruned seeds be T_0, the seedlings that emerged from intact seeds be H_0, p_i^0 is the probability of seedling establishment of intact seeds, and p_c^0 is the total probability of seedling establishment of pruned seeds and pruned roots:

$$p_i^0 = H_0/N_0 \tag{5}$$

$$p_c^0 = \frac{T_0 + S_0}{x_0 + M_0 x_0/(x_0 + N_0)} \tag{6}$$

Detailed seed fates (i.e. seedling establishment, seed consumption and seed death) of relocated seeds with coded tags were also analyzed. This analysis does not include pruned roots which cannot be linked to their original coded seeds. Let the number of seedlings, eaten seeds and dead seeds be n_1, n_2 and n_3, respectively; $n = n_1 + n_2 + n_3$; and let the probabilities of seedling establishment, seed consumption and seed death be p_1, p_2, and p_3, respectively:

$$p_1 = n_1/n \tag{7}$$

$$p_2 = n_2/n \tag{8}$$

$$p_3 = n_3/n \tag{9}$$

Simulated seed pruning and embryo-removal experiments

In September 2008, we conducted three experiments to study the effect of simulated "embryo removal" and root-pruning on seed germination and seedling establishment of *P. kerrii* in a rainforest at the Xishuangbanna Tropical Botanical Garden. During the experiments, in order to simulate seed hoarding of rodents, seeds were placed under leaf litter along a grid of 10 × 10 cm in the plot. Seeds were protected using the same wire mesh enclosures as

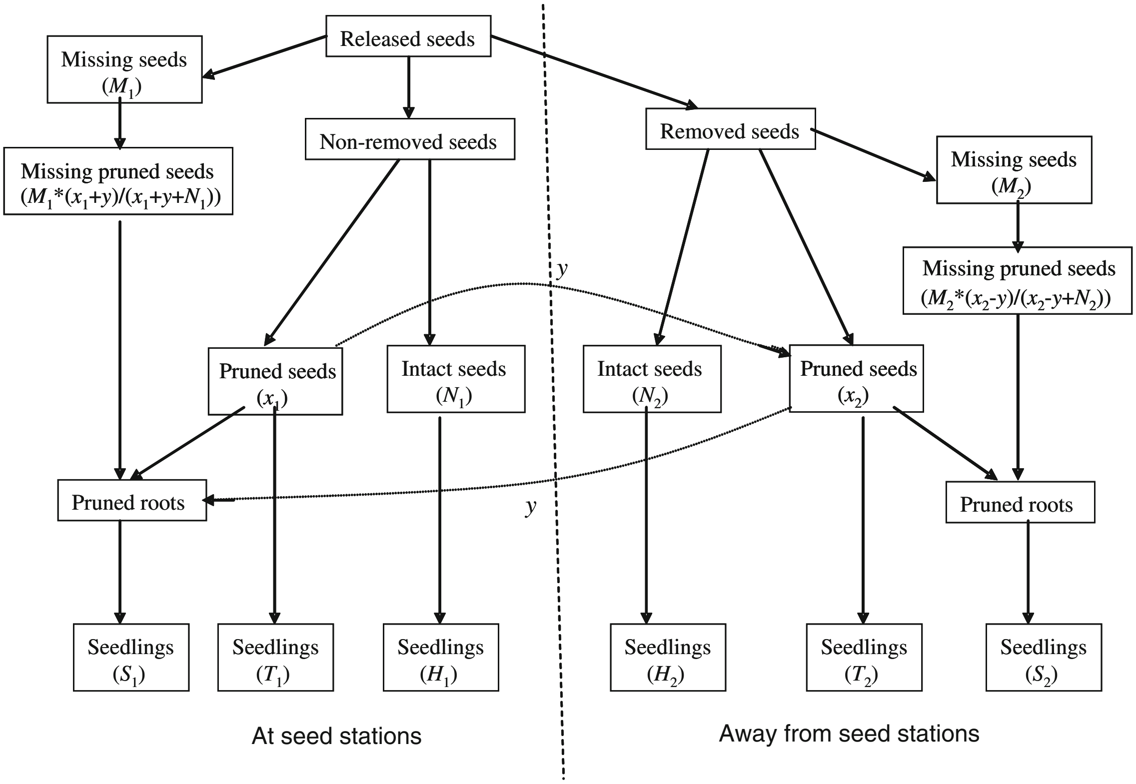

Fig. 1 Illustrations of seedling establishment of intact seeds, pruned seeds and pruned roots of *Pittosporopsis kerrii* at seed stations and away from seed stations. This figure was used for estimating probability of seedling establishment in Fig. 3 according to Eqs. 1, 2, 3, 4, 5 and 6. The *dashed lines* denote the number of seeds pruned at seed stations and transported to sites away from the seed stations by rodents, resulting in potential pruned roots at seed stations. The number of missing pruned seeds was estimated by assuming the proportion of pruned seeds in missing seeds was same as that in relocated pruned seeds and intact seeds

above which prevented vertebrates from accessing the seeds. The first experiment tested the differences in the rate of nutrient loss (as measured by dry weight of seeds) between pruned seeds (pruned one or two times) and intact seeds. Seeds were divided into three groups: intact seeds (control group), seeds that had their seminal root pruned once 2 weeks after seed placement (once-pruned group), and seeds with their seminal root pruned twice 2–4 weeks after seed placement (twice-pruned group). Fifty control seeds were taken back to the laboratory at weeks 1, 2, 3, 4, 6, 8, 10 and 12 after seed placement. A sample of pruned seeds ($n = 25$–37) were taken back at weeks 4, 6, 8, and 10. Some twice-pruned seeds ($n = 21$–26) were taken back at weeks 6, 8, 10 and 12. All seeds sampled were randomly selected. These seeds were dried and weighed in the laboratory. The numbers of decomposed seeds (determined by physical evidence that seed were developing indigestible taproots) of each treatment group were also counted.

The second experiment tested the differences of seed germination and seedling establishment between intact seeds and "embryo-removed" seeds. We removed most of the embryo except for some fragments of cotyledon by notching a hole close to the radicle. Then, 50 intact seeds and 50 "embryo-removed" seeds were taken back to laboratory for germination trials at weeks 1, 2 and 4. A total of 100 embryo-removed seeds were used for checking taproot or seedling establishment.

A third experiment tested the differences of seedling establishment among intact seeds, pruned seeds (with their seminal roots being removed) and pruned seminal roots. Seminal roots were removed from 18–50 seeds at weeks 2, 4, 6, 8 and 10 after seed placement. The pruned seminal roots were kept at the same places where their seeds were initially placed, while pruned seeds were placed under leaves of different places nearby. A total of 150 intact seeds served as the control group and were placed under leaves. The seedling establishment was checked in January, March, July and September of the following year.

Hoarding behavior experiments

In January and October 2009, we studied the hoarding behavior of four key rodent species (*N. confucianus*, *R. flavipectus*, *M. surifer*, *N. fulvescens*) and their behavior of seminal root pruning by using four semi-natural enclosures (L × W × H = 10 × 10 × 1.5 m) (Cheng et al.

2005). To prevent rodents from escaping from the enclosure and to prevent other animals from entering it, the wall of the enclosure was built using concrete and buried under the ground surface to 0.5 m in the soil. The top of the enclosure was covered with a plastic cloth to prevent rain during the experiment. We captured rodents in the field by using live traps (L × W × H = 14 × 14 × 30 cm) made of steel wire mesh (Chang et al. 2009), and baited with fresh peanuts. The species, body weight and reproductive status of captured animals were recorded before being taken back to laboratory for enclosure experiments. Pregnant females or juveniles were immediately released in the field. All adult animals were kept individually in a cage (40 × 30 × 25 cm) and provided with adequate food, water and nest material. A photoperiod cycle of 12:12 h (light:dark) was maintained. During the experiments, one animal was placed in the enclosure and observed for three consecutive days. The animal was provided with laboratory food on the first day in order to ease adaptation to the new environment. On the second and last days, ten germinated and non-germinated seeds, respectively, were placed in the enclosure. Seeds were tagged with plastic markers as described above. Rodents were provided with one seed station in the middle of each enclosure, and one underground nest in the corner of the enclosure. Seeds were classified as eaten seeds, pruned seeds or intact seeds based on the patterns of use by the rodents.

Statistics

Chi-square was used for testing differences in the probabilities of taproot or seedling establishment, seed consumption, and seed death (Jansen et al. 2006; Xiao et al. 2009). The Mann–Whitney U test was used to identify the significant differences in probability of root-pruning by the four rodent species with regard to germinated and non-germinated seeds. The Cox regression was used for comparing the differences of mean survival (before eaten, decomposed, or seedling establishment) time between pruned seeds and intact seeds in field conditions.

Results

Live-trapping results revealed that *N. confucianus* were the predominant rodent species in the study site; the other three species (e.g., *M. surifer; R. flavipectus*; *N. fulvescens*) were commonly seen. By using semi-natural enclosures, we found that all four rodent species tested (Fig. 2e–h) showed significantly higher frequency of seed pruning behavior (e.g., root-pruning) of germinated seeds than of non-germinated seeds (Fig. 2a–d; all $P < 0.001$ or $P < 0.01$). We did not observe any of these four species show embryo-removal behavior during the enclosure test. The predominant behavior of *M. surifer* was scatter-hoarding, whereas *R. flavipectus* mostly demonstrated larder hoarding of both germinated and non-germinated seeds. *N. confucianus* and *N. fulvescens* showed some scatter hoarding, but exhibited more larder hoarding.

Our seed releasing experiment in natural conditions shows that the probability of total seedling establishment of pruned seeds and pruned roots was significantly higher than those of intact seeds for all seeds ($X^2 = 13.715$, $P < 0.001$) and for removed seeds ($X^2 = 103.588$, $P < 0.001$), but slightly lower than that of intact seeds for non-removed seeds ($X^2 = 4.979$, $P = 0.026$) (Fig. 3). During the survey process, 100 and 19 seedlings were found to emerge from pruned roots and pruned seeds, respectively, at seed stations, making up 22.9 and 4.3% of all seedlings ($n = 437$) which emerged from non-removed seeds, while 10 and 44 seedlings were found to emerge from pruned roots and pruned seeds, respectively, away from the seed station, making up 13.9 and 61.1% of all seedlings ($n = 72$) which emerged from removed seeds.

Detailed seed fates of relocated seeds with coded tags (not including pruned roots) are shown in Fig. 4. For all seeds, removal by rodents significantly reduced the probability of seedling establishment of *P. kerrii* seeds ($X^2 = 566.270$, $P < 0.001$) and the probability of seed death ($X^2 = 409.676$, $P < 0.001$); but significantly increased the probability of seed consumption ($X^2 = 1213.252$, $P < 0.001$) of removed seeds (Fig. 4b). For all seeds, root-pruning by rodents showed no significant effect on probabilities of seedling establishment ($X^2 = 2.020$, $P = 0.155$), seed consumption ($X^2 = 0.009$, $P = 0.925$) and seed death ($X^2 = 2.118$, $P = 0.146$) of pruned seeds (Fig. 4f). However, for non-removed seeds, root-pruning significantly reduced the probability of seedling establishment ($X^2 = 16.618$, $P < 0.001$), but increased the probability of seed consumption ($X^2 = 18.143$, $P < 0.001$) of pruned seeds; the difference in probability of seed death between pruned seeds and intact seeds was not significantly ($X^2 = 0.834$, $P = 0.361$) (Fig. 4c). For removed seeds, root-pruning significantly increased the probability of seedling establishment ($X^2 = 89.574$, $P < 0.001$) and the probability of seed death ($X^2 = 68.256$, $P < 0.001$), but reduced the probability of seed consumption ($X^2 = 164.403$, $P < 0.001$) of pruned seeds (Fig. 4d). During the seed releasing experiments, we found 707 seeds (303 intact seeds and 404 pruned seeds) were cached after removed from seed station, but 235 seeds (109 intact seeds and 126 pruned seeds) missed in the following survey process, and ultimately 72 seeds (18 intact seeds and 54 pruned seeds) establish seedlings in the caches sites. By analyzing the seed fates of relocated cached seeds, we found root-pruning significantly increased the probability of seedling

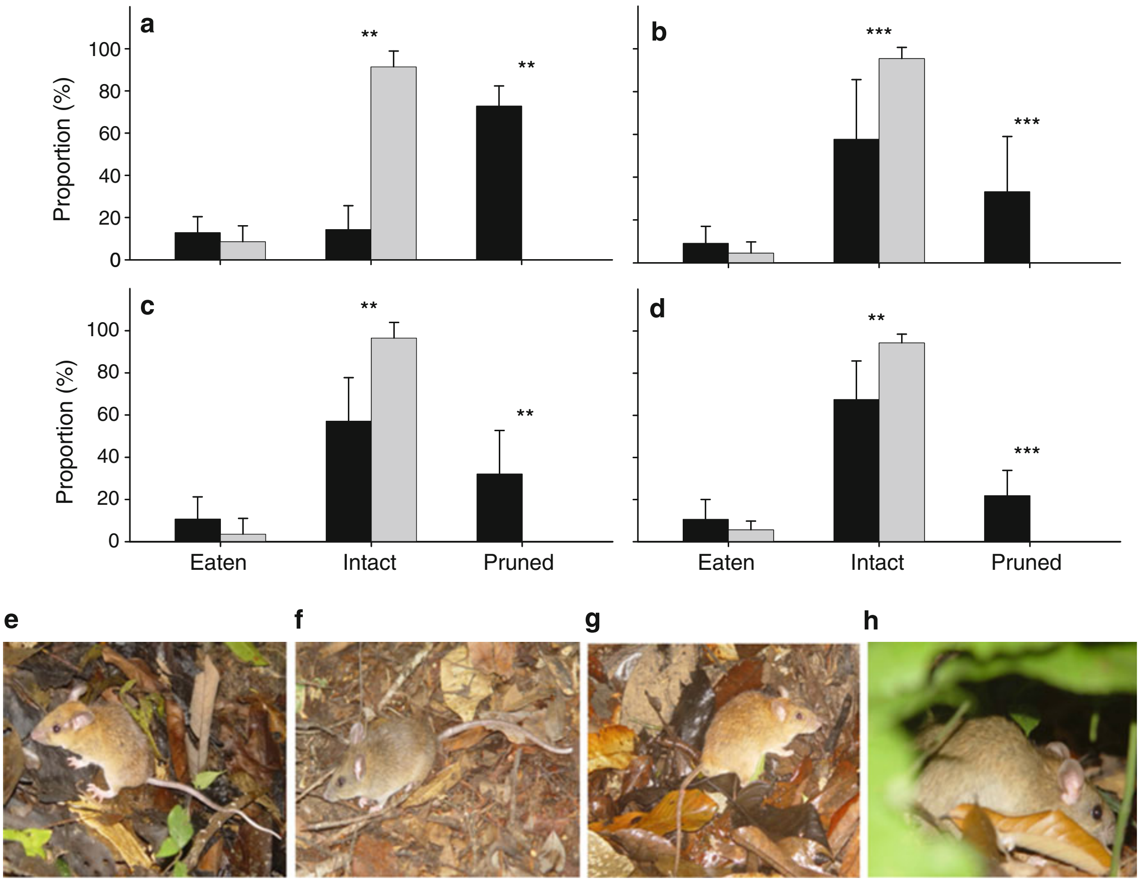

Fig. 2 The differences in mean ± SD proportions of eaten seeds, intact seeds and pruned seeds by the four sympatric rodent species between germinated seeds and non-germinated seeds of *P. kerrii* in enclosures. *Black bars* germinated seeds, *gray bars* non-germinated seeds. **a**, **e** *Maxomys surifer* ($n = 7$); **b**, **f** *Niviventer confucianus* ($n = 11$); **c**, **g** *Niviventer fulvescens* ($n = 7$); **d**, **h** *Rattus flavipectus* ($n = 8$). $^{**}P < 0.01$, $^{***}P < 0.001$

establishment ($X^2 = 9.099$, $P = 0.003$) and the probability of seed death ($X^2 = 10.391$, $P = 0.001$), but reduced the probability of seed consumption ($X^2 = 22.283$, $P < 0.001$) of pruned seeds (Fig. 4e).

Based on data of intact seeds or pruned seeds of non-removed and removed seeds in 2007 and 2008, the probability of seedling establishment was significantly and negatively correlated with probability of seed consumption ($r = -0.880$, $P = 0.004$, $n = 8$). The same pattern was present when we analyzed data of intact and pruned seeds in all stands in 2007 and 2008 ($r = -0.981$, $P < 0.001$, $n = 11$).

The probabilities of seedling establishment of roots that we artificially pruned from the germinated seeds at weeks 2, 4 and 6 were significantly lower than those of the intact seeds; however, the probabilities at weeks 8 and 10 showed no significant differences (Fig. 5e). The probabilities of seedling establishment of simulated pruned seeds at weeks 2, 6, 8 and 10 were significantly lower than those of the intact seeds, but the probability at week 4 showed no significant difference from intact seeds (Fig. 5f). The total probability of seedling establishment of pruned seminal roots and pruned seeds at week 2 was significantly lower than that of intact seeds; however, the probabilities at weeks 4, 6, 8 and 10 showed no significant differences from those of intact seeds (Fig. 5g).

Both probabilities of taproot and seedling establishment of simulated “embryo-removed” seeds were significantly lower than those of the intact seeds (Fig. 5h). However, the probabilities of taproot (89%) and seedling emergence (39%) of embryo-removed seeds were still quite high, suggesting that the cotyledon also has a high capacity for natural regeneration. The probability of germination of the “embryo-removed” seeds (0%, $n = 50$) was significantly lower than that of intact seeds (80%, $n = 50$) 1 week after being placed under leaf litters. However, the difference between the “embryo-removed” seeds (88%, $n = 50$) and intact seeds (96%, $n = 50$) was not significant at 2 weeks. Thus, the simulated “embryo removal” can delay seed germination for about 1 week. In addition, we found 51.2% of embryo-removed seeds ($n = 82$) produced 2–4 seminal roots (Fig. 5d). The simulated “embryo removal” did not

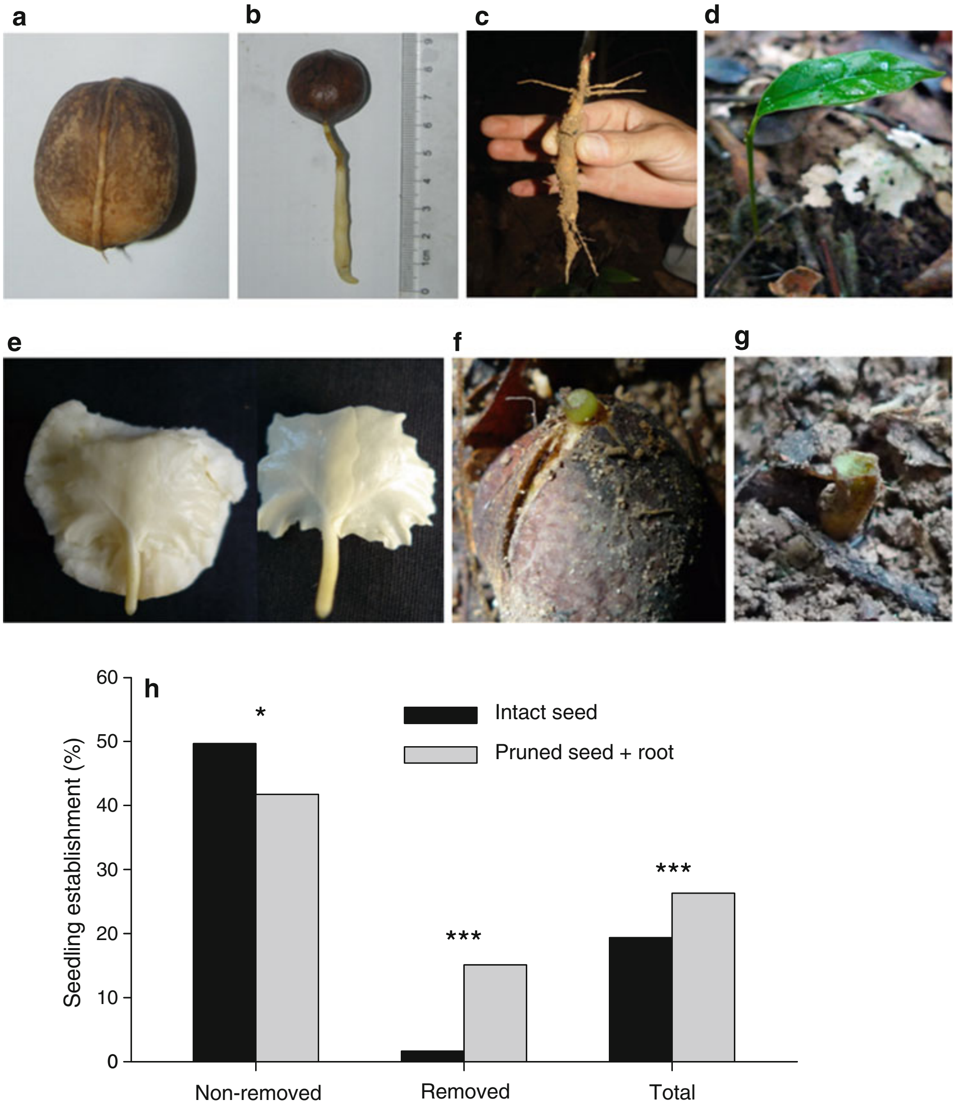

Fig. 3 Seed germination properties of *P. kerrii* seeds and effects of seed pruning or removal on seedling establishment by rodents in the Xishuangbanna tropical forest of Yunnan Province, China. **a** Intact seed, **b** germinating seed, **c** taproot, **d** seedling, **e** endosperm, cotyledon and radicle of the seed, **f** pruned seed by rodent, **g** pruned root by rodents, **h** differences in proportions of seedling establishment between intact seeds and sum of pruned seeds and pruned roots for non-removed, removed and total seeds. ***$P < 0.001$. The proportion of seedling establishment was estimated according to Fig. 1 and Eqs. 1, 2, 3, 4, 5 and 6

significantly reduce the probability of germination, but it reduced the probability of taproot establishment slightly, and seedling establishment considerably.

As shown in Fig. 6, the dry weight of pruned seeds with seminal roots pruned either once or twice decreased more slowly than that of intact seeds. Hence, pruning of seeds can reduce nutrient loss for about 2–4 weeks as compared to intact seeds. The probability of seed decomposition was also significantly reduced as compared to intact seeds; the decomposing of seeds can be delayed for about 4–5 weeks. These results suggest that seminal root pruning by rodents can significantly delay nutrient loss and seed decomposition. This behavior is likely a strategy used by rodents to increase food storage. Field studies on mean survival time (before eaten, decomposed, or seedling establishment) of pruned seeds and intact seeds also support these observations. In field conditions, the mean survival time (mean ± SE) of pruned seeds was 132 ± 4.1 days ($n = 559$). This was significantly longer than that of intact seeds (84 ± 1.6 days, $n = 3{,}041$; Cox regression, Wald = 87.581, $df = 1$, $P < 0.001$).

Discussion

Rapid germination of non-dormant seeds is used by tree species to reduce seed losses by rodents (Chang et al. 2009; Fox 1982; Hadj-Chikh et al. 1996), whereas in response, embryo-removal is used by some rodent species to stop or delay seed germination (Steele 2008; Steele et al. 2001; Xiao et al. 2009). Previous studies have demonstrated that, in general, embryo removal causes the failure of natural regeneration of seeds (Mendoza and Dirzo 2009; Steele 2008; Xiao et al. 2009). Multi-seeded acorns from some oak trees can germinate and develop into healthy seedlings when one seed is destroyed by squirrels (McEuen and

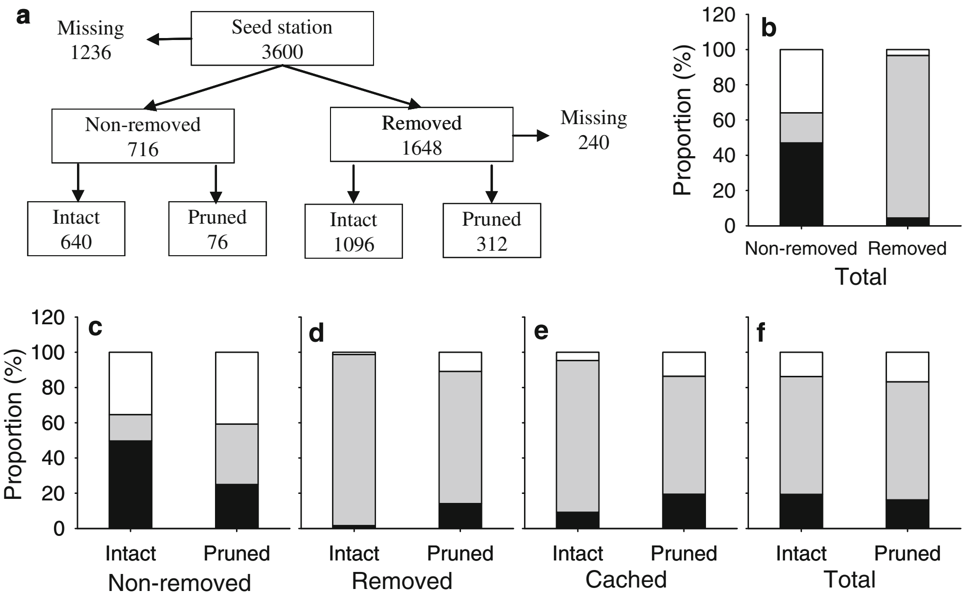

Fig. 4 Effects of seed removal and root pruning on proportions of seedling establishment, seed consumption and seed death of all relocated *P. kerrii* seeds with coded tags (excluding pruned roots of unknown coded seeds). Shown are number of seeds removed and pruned (**a**); and proportions of seedling establishment (*black bars*), seed consumption (*grey bars*) and seed death (*white bars*) of removed and non-removed seeds (**b**), of intact or pruned seeds that were not removed (**c**), of intact seeds or pruned seeds that were removed (**d**), of intact seeds or pruned seeds that were cached (**e**), of total intact seeds and pruned seeds (**f**). Proportions of seedling establishment, seed consumption and seed death are calculated based on Eqs. 7, 8 and 9

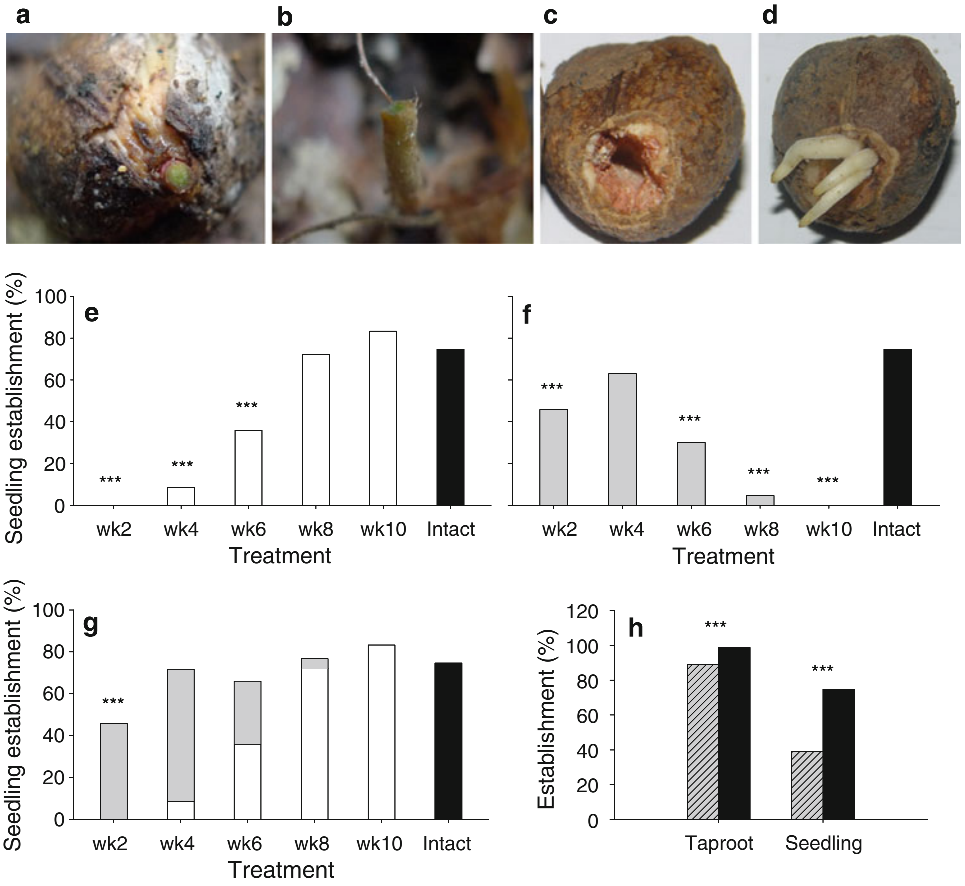

Fig. 5 Effects of simulated embryo removal or seminal root pruning on seed germination and seedling establishment of *P. kerrii*. **a** Simulated pruned seed, **b** simulated pruned root, **c** simulated embryo removed seed (cotyledon fragments *left*), **d** germinations (showing multiple seminal roots) of simulated embryo removed seed, **e** difference in proportion of seedling establishment between intact seeds and pruned roots, **f** difference in proportion of seedling establishment between pruned seeds (after seminal roots being pruned) and intact seeds, **g** difference in proportion of seedling establishment between intact seeds and sum of pruned roots and pruned seeds, **h** difference in proportion of taproot or seedling establishment between intact seeds and “embryo-removed” seeds. *Black bars* intact seeds, *white bars* pruned roots, *gray bars* pruned seeds and *hashed bars* embryo-removed seeds. ***$P < 0.001$

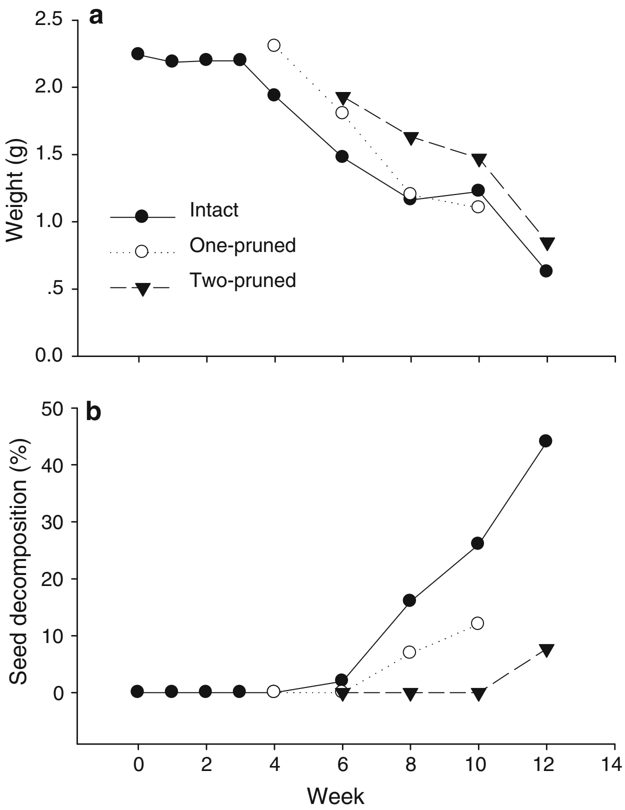

Fig. 6 Differences in dry weight of seeds (**a**) and proportion of seeds decomposed (**b**) after their seminal roots were pruned once at 2 weeks after germination, and pruned twice at 2–4 weeks after germination, as compared to intact seeds

Steele 2005; Steele 2008). However, the frequency of multi-seeded acorns is very low (less than 3% in oak populations), and embryo excision was likely to be a selective force for maintaining multi-seeded acorns within oak populations (McEuen and Steele 2005). Besides, this trait in oaks may also be maintained by insects (e.g., *Curculio*), not solely by squirrels (McEuen and Steele 2005; Steele 2008).

In this study, we demonstrate that seminal root pruning, instead of embryo removal, is commonly used by the sympatric rodents to increase the storage time of seeds by slowing germination process of *P. kerrii* seeds. Most intact seeds of *P. kerrii* germinated quickly after falling to the ground, and developed a taproot soon thereafter, but germination process or taproot appearance was delayed for several weeks due to root-pruning by rodents. We also found that the rodents pruned the seminal roots of the germinated seeds at seed stations even though they did not remove the seeds immediately during both field and enclosure experiments. The reason why rodents prune seeds at seed stations (non-removed) is that not all pruned seeds are successfully removed by rodents, or they are in the process of seed removal. This observation indicates that rodents show a tendency to prune roots of germinated seeds before they are dispersed. In addition, we found 16 seeds experienced pruning twice by rodents, suggesting multiple seed pruning also exists. This observation supports our first hypothesis that seed pruning is used by rodents to increase the storage time of non-dormant seeds. It is notable that all the four sympatric rodent species tested showed seed pruning behavior, indicating that seed pruning behavior may be widely adopted by many rodent species in tropical regions.

It is not clear why rodents in the tropical forests prefer pruning seminal roots over excising embryos of seeds. The seed coat of *P. kerrii* seeds is quite thin; obviously it is not an effective barrier for preventing embryo removal by rodents. Our simulated experiment, however, demonstrated that *P. kerrii* seeds are very tolerant of "embryo removal"; incomplete embryo-removed seeds easily germinate or establish as normal seedlings. The dicotyledon of *P. kerrii* seed is very large, and our simulated embryo removal did not remove all the cotyledon embedded in the endosperm. We found that seminal roots that emerged from the embryo still had a high capacity of regeneration. It is obvious that embryo-removal is much harder than root pruning for rodents. In addition, embryo-removal may also increase the risk of fungi infection cmpared with root pruning. This may explain why rodents adopt the simple strategy of seminal root pruning in slowing down the seed germination process.

Our study reveals that both pruned seeds and pruned roots of germinated seeds of *P. kerrii* can develop into normal seedlings under both experimental and natural conditions. This suggests that *P. kerrii* seeds are highly tolerant to seminal root pruning, and both pruned seeds and pruned roots have high regeneration capacity. Hence, both the dicotyledon and the taproot possess the capacity for regeneration. To our knowledge, this is the first report that plant seeds could successfully escape seed predation after embryo removal and seed pruning by adopting a tolerance strategy with a high regeneration capacity of both pruned seeds and roots. This observation supports our second hypothesis that a high regeneration capacity may be an adaptive strategy adopted by plants to counter seed predation by rodents. The seed pruning behavior in rodents and the high regeneration capacity of pruned seeds or roots in *P. kerrii* seeds observed in this study are likely novel adaptation strategies adopted by seeds and rodents, respectively. We predict that this phenomenon may exist widely in nature.

Mutualism in a competition or predation system may benefit both competitors or prey and predator (Zhang 2003; Zhang et al. 2005). It is obvious that the high regeneration capacity of *P. kerrii* seeds, by increasing the survival capacity of seeds pruned by rodents, has significantly enhanced the mutualism between seeds and rodents. Compared to excising embryos of seeds, the strategy of pruning seminal roots by rodents may be advantageous to both seeds and rodents. Seminal root pruning may be better than embryo removal in reducing fungi infections and seed decay in wet tropical conditions, and may therefore aid long-term food storage (good for rodents), and also does not decrease seedling establishment (good for seeds). It is notable that the improved mutualism is likely caused by adaptation of both plants (high regeneration capacity) and rodents (root pruning).

Seeds of *P. kerrii* were observed to germinate very rapidly after seed fall, often within 1 week, which seems to be a common strategy adopted by many tree species in tropical forests (Jansen et al. 2006; Vazquez-Yanes and Orozco-Segovia 1993; Xiao et al. 2009, 2010). However, instead of developing into seedlings, the germinated seeds of *P. kerrii* first developed into indigestible taproots and then decomposed within 2–3 months. We found that only one taproot and no seedlings were destroyed by rodents. The rapid decomposition of the seed body might contribute to the survival of the taproots and seedlings of *P. kerrii* by reducing the attractiveness of the seeds to rodents. To test this, we observed 640 taproots and 509 seedlings established from 3,600 tagged seeds in our field study. We found that the appearance of the taproot allowed seeds to escape predation by rodents. This is likely another evolutionary strategy developed by *P. kerrii* seeds to withstand seed predation. Taproots seem to mimic dormant seeds as they have the ability to develop into seedlings many months after they form. Taproot and rapid seed decomposition are two resistance traits of *P. kerrii* seeds for countering seed predation by rodents.

We found that the probability of seedling establishment of *P. kerrii* seeds at seed stations (non-removed seeds) was higher than removed seeds that were dispersed away from seed stations (Fig. 4b). It was a masting year in 2008 when a majority of seedlings emerged from seed stations. Seeds disappeared slowly, and many seeds of *P. kerrii* germinated and developed into taproots or seminal roots at seed stations before they were removed or eaten by rodents. Both taproots and pruned roots that remained in the soil can develop into normal seedlings under favorable conditions. The probability of seed consumption of removed seeds by rodents was much higher than that of non-removed seeds (Fig. 4b), resulting in a lower probability of seedling establishment for removed seeds. The higher probability of seedling establishment for seeds at seed stations indicates that natural regeneration of *P. kerrii* depends on successful seedling establishment of both dispersed and non-dispersed seeds. This is probably because *P. kerrii* is a dominant shrub species in the tropical forests and its seedlings are shade tolerant. In tropical forests, competition for space is very intense among plants. A high density of seedling establishment under parent trees will prevent invasions of other shrub or tree species. Though there is a low probability of seedling establishment of removed seeds, these seeds are likely to be essential for occupying empty spaces or for colonizing new habitats.

Non-removed seeds showed a higher probability of seed death than did removed seeds (Fig. 4b). Seed death was caused by factors other than the rodents (e.g., environmental stresses of rain, light, and desiccation or biological stresses of insect predation or fungal infection) (Lin Cao, personal observation). Removed seeds were mostly cached under leaf litter in a wet microclimate, and thus may have had a lower probability of death. We found root pruning increased the seedling establishment of removed seeds (Fig. 4d), but decreased the seedling establishment of non-removed seeds (Fig. 4d). This was caused by the difference in seed consumption by rodents between intact seeds and pruned seeds of removed or non-removed seeds. Rodents showed different feeding preferences for intact seeds or pruned seeds at seed stations and away from seed stations. As shown in Fig. 6, pruned seeds have a higher nutritional value and more long-term storage value than intact seeds. Much of the nutritional value of the intact seeds might have been transported into the roots. This may explain why rodents prefer to eat more intact seeds at the cache sites (Fig. 4c).

In summary, we found that rodents adopted a root-pruning strategy to extend the storage time of seeds, and that non-dormant seeds of *P. kerrii* possessed the following traits that allowed seeds to better escape seed predation by rodents: rapid germination, seed decomposition, dormant taproots, and high regeneration capacity of pruned roots, pruned seeds or even embryo-removed seeds. The anti-predation traits of *P. kerrii* Craib seeds may be the result of evolutionary adaptation to the intensive seminal root pruning and seed predation of germinated seeds by rodents. The high regeneration capacity of plant seeds is a very successful strategy to balance the effect of predation and dispersal on seed survival, and thus promotes mutualism as well as species coexistence between seeds and rodents in the tropical forests.

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