Weak olfaction increases seed scatter-hoarding by Siberian chipmunks: implication in shaping plant–animal interactions

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Scatter-hoarding of seeds by animals plays an essential role in seed dispersal of plants and in shaping plant–animal interactions in forest ecosystems, but the function of scatter-hoarding behavior is still unclear. We hypothesize that weak olfactory cues between seeds and scatter-hoarding animals would increase scatter-hoarding. Using a rodent–plant system of Siberian chipmunks *Tamias sibiricus* and Korean pines *Pinus koraiensis*, we tested the effects on seed scatter-hoarding intensity by measuring and modifying the seed odor intensities and the abilities of the animals to detect seed odor. Siberian chipmunks seemed to scatter-hoard more seeds with weaker odor signals, and Siberian chipmunks with reduced olfactory ability scatter-hoarded more seeds, supporting our hypothesis. Our studies suggest that olfaction may have played an important role in the evolution of the strength of seed odor and scatter-hoarding behavior of animals, and in shaping plant–animal interactions.

Seeds can be dispersed by a variety of animals (Vander Wall 1990, Tsujino and Yumoto 2009, Yi et al. 2012, González-Varo et al. 2013, Krebs 2014). Among those, food-hoarding animals play a key role in seed dispersal of various plants bearing large seeds (Vander Wall 1990, Steele et al. 2005, 2015, Hirsch et al. 2012). Animals often use scatter-hoarding and/or larder-hoarding to store food items (Vander Wall 1990). Scatter-hoarding animals usually store seeds in small, widely scattered and shallow caches (Steele et al. 2011, Yi et al. 2012, Neuschulz et al. 2015), which has been recognized to play an essential role in seedling establishment of large-seeded trees (Vander Wall 1990, Jansen et al. 2014). Larder-hoarding behavior would not benefit plant regeneration because larder-hoarded seeds are less likely to establish seedlings. Thus, there will be a stronger component of mutualism in the relationship between seeds and scatter-hoarding animals (Vander Wall 1990).

Scatter-hoarding animals need to invest more energy than larder-hoarders to establish, manage and even recover their scattered caches (Vander Wall 1990, Leaver 2004, Yi et al. 2012). Because of these seemingly negative drawbacks, it has long been a puzzle why many groups of animals exhibit the behavior of scatter-hoarding or use a combination of larder-hoarding and scatter-hoarding (Vander Wall 1990, Clarke and Kramer 1994, Preston and Jacobs 2001, Vander Wall et al. 2005). It has been speculated that if animals are unable to defend their hoarded food items, then an alternative strategy of scatter-hoarding will be adopted (Dally et al. 2006, Luo et al. 2014). This strategy assumes that scattering food items over many widely spaced caches spreads risk of pilferage by competitors (Devenport et al. 2000, Dally et al. 2006). Reciprocal cache pilferage and pilferage avoidance are found to influence hoarding patterns of animals (Vander Wall and Jenkins 2003, Dally et al. 2006, Leaver et al. 2007, Rusch et al. 2013, Steele et al. 2014), and are believed to drive evolution of scatter-hoarding behavior of food-hoarding animals.

Recently, seed odor and olfactory ability of animals have been suggested to affect cache loss or pilferage by animals (Vander Wall 1990, Briggs and Vander Wall 2004, Taraborelli et al. 2009, Hollander et al. 2012, Paulsen et al. 2013). Seeds that emit strong odor are more likely to be pilfered from caches by competitors (Hollander et al. 2012, Paulsen et al. 2013). Natural selection is likely to produce seeds with weak odor (Hollander et al. 2012), which is expected to reduce predation by animals. However, it has not been tested how seed odor signals and olfactory abilities of animals affect scatter-hoarding strategy of animals. Scatter-hoarding animals are known to retrieve their caches by both spatial memory and olfactory ability, while pilferers rely mainly on olfactory and random search for finding caches (Vander Wall 1990, 1991, Jacobs 1992). Therefore, we proposed a hypothesis that weak olfaction between seeds and scatter-hoarding animals would increase scatter-hoarding.

Siberian chipmunks *Tamias sibiricus* are widely distributed in northeastern China, and they are known to scatter-hoard seeds of a variety of tree species (Yi et al. 2012, 2013). Korean pines *Pinus koraiensis* produce large, high nutritional

seeds (about 0.7 g in mass), which are ideal food for the local hoarding animals, such as *T. sibiricus*. As the dominant species of broad-leaved Korean pine forest, *P. koraiensis* is almost absolutely dependent on the scatter-hoarding animals for their natural regeneration (Zong et al. 2009). In this study, by altering seed odor signals of *P. koraiensis*, removing bilateral olfactory bulbs of *T. sibiricus*, and observing *T. sibiricus* with different olfactory abilities, we investigated how olfaction affects cache pilferage and scatter-hoarding patterns of *T. sibiricus* so as to test our hypothesis. We have several predictions: 1) seeds with stronger odors are more likely to be pilfered; 2) seeds with stronger odors are less likely to be scatter-hoarded; 3) chipmunks with reduced olfactory ability will pilfer less seeds; 4) chipmunks with reduced olfactory ability will scatter-hoard more seeds. To our knowledge, this is the first study to test how olfaction between seeds and animals affects scatter-hoarding intensity of animals.

Methods

Study site and animal handling

The study was conducted from September to October 2014 in the Dongfanghong Forestry Center (mean elevation of 750 m, 45°58′N, 129°08′E) in the Dailing District, Yichun City, Heilongjiang Province, northeast China. To trap Siberian chipmunks for enclosure experiments, steel-framed live-traps ($9 \times 10 \times 25$ cm) baited with peanuts and carrots, were placed in forests at 5-m intervals along four transects at 8:00 am. All traps were pre-baited for one day and protected from predators by wrapping with steel mesh. Traps were checked twice daily in the early morning and late afternoon for six consecutive days. Trapping stopped when encountering bad weather (e.g. heavy rainfall). The captured Siberian chipmunks were transported by vehicle to the animal housing room. Chipmunks were kept in frame cages ($30 \times 40 \times 50$ cm) individually at a range of temperatures (day 15–20°C, night 10–15°C) and natural photoperiod (about 14 light hours). They were provided with nests, carrots, peanuts, seeds and water ad libitum. No animals died during field trapping.

Enclosures

We conducted behavioral experiments in sixteen separate enclosures (10×10 m) established in an open, non-forested area (Yi et al. 2012). Brick enclosures were covered with concrete and are extended about 2.5 m above ground and 0.5 m below the soil surface. The top of the enclosure was covered with plastic nettings to prevent access to predators. An artificial burrow was provided at one corner of each enclosure, and an arena was established at the center of each enclosure. The enclosures were divided into two sections for both animal scatter-hoarding and pilfering experiments. In the semi-natural enclosures, the ground was filled with forest soil and had dwarf grasses growing over more than 95% of the ground area (Supplementary material Appendix 1 Fig. A1A). In the paved enclosures, the ground was paved with bricks to create 64 shallow pits (brick length × width × depth: $24 \times 12 \times 6$ cm) evenly spaced in an 8×8 grid (Supplementary material

Appendix 1 Fig. A1B). A piece of iron sheet was laid beneath each of the pits to prevent from burrowing of animals. These small pits were filled with fine sand to allow scatter-hoarding animals to bury seeds. We also established artificial caches in these shallow pits to study cache pilferage.

Experiment 1

Effects of increased seed odor signals on cache pilferage and scatter-hoarding

As seed moisture regulates volatile emission from seeds (Vander Wall 1998, Paulsen et al. 2013), we aimed to increase seed odor signals by soaking seeds of *Pinus koraiensis* in distilled water for 0, 2 and 4 h. Seeds were not totally immersed to ensure seed respiration, but were regularly stirred. To test if water uptake increased with soaking times, we randomly selected 10 seeds of *P. koraiensis* of each treatment and measure their fresh masses. To see if seed odor signals increased with soaking times, six seeds of *P. koraiensis* of each soaking treatment were initially sealed in three 10-ml gas chromatography (GC) headspace vials and stored at 4°C for five days before headspace analyses (two seeds per vial). Odorous compound analyses were performed using a headspace autosampler GC-MS in the Environmental Protection Dept of Jiangxi Province (Nanchang, China). The odorous compounds were separated using GC on a HP-5MS column (30 m length, 0.32 mm internal diameter, 0.25 µm film thickness) running a temperature program (4 min hold at 40°C, 5°C per min to 100°C, 10°C per min to 200°C and 2 min hold; helium carrier gas at constant flow rate of 1 ml per min). The main volatile compounds were identified from the NIST (Natl Inst. of Standards and Technology, Gaithersburg, MD, USA) mass spectral database. Seed odor intensity was expressed as abundance of ions of a given compound.

To see if seed odor signals affect cache pilferage rates, we established 15 artificial caches in the shallow pits for each soaking treatment in each paved enclosure. Because Siberian chipmunks often establish shallow caches (less than 2 cm in depth) containing single seed, one seed of *P. koraiensis* was buried 2-cm deep in each pit to create 45 artificial caches to allow *Tamias sibiricus* to pilfer freely. We randomly scattered 45 artificial caches in 64 pits because this deployment avoided learning of a set formation of caches when chipmunks pilfer seeds. The sand of the remaining nineteen empty pits was also mixed evenly and considered as sham caches. Sand in all pits was smoothed to avoid discrimination between real and sham caches through visual cues by chipmunks. One animal was released into each enclosure at 9:00 am on the day of the experiment. We checked cache pilferage rates of each treatment 4 h after introduction of animals. In total, 12 chipmunks (4♀, 8♂) were tested to see the effects of seed moisture on cache pilferage rates.

To test if seed moisture affect scatter-hoarding intensity (the amount of scatter-hoarding) of Siberian chipmunks, we provided each of 8 (3♀, 5♂) Siberian chipmunks with 20 *P. koraiensis* seeds of each soaking treatment at the center of the paved enclosures at 9:00 a.m. Seeds of each soaking treatment was numbered specifically for identification by using pencil. At 17:00 p.m., we checked how many seeds of each treatment were scatter-hoarded in the shallow pits by Siberian chipmunks. The numbers of scatter-hoarded seeds

of each treatment were compared to see the influence of seed soaking on scatter-hoarding of Siberian chipmunks.

Experiment 2

Effects of decreased seed odor signals on cache pilferage and scatter-hoarding

To mask odor signals of seeds, we used Carmars aerosol colorless peelable hub spray membrane to coat seeds of *P. koraiensis*. Seeds were randomly selected and divided into three groups. The first group was treated as control (uncoated). Seeds of the second group were spread evenly on the ground and only half of each seed was evenly coated with spray membrane twice (partially-coated). For the third group, the whole seed was coated with spray membrane twice (wholly-coated). Membrane-coated seeds were kept at room temperature for two weeks to minimize the weak odorous effects of the spray membrane coating. To see if coating decreased seed odor signals, three seeds of each coating treatment were randomly selected and sealed individually in a 10-ml gas chromatography headspace vial. After stored at 4°C for 24 h, headspace analyses were performed in the same procedure as mentioned above.

Later, we established 15 artificial caches for each seed-coating treatment in each paved enclosure. Following the same procedure as mentioned in experiment 1, cache pilferage rates of each coating treatment were checked. In total, 16 chipmunks (9♀, 7♂) were tested to see the effects of seed coating on cache pilferage rates. Similarly, we provided each of 9 (4♀, 5♂) Siberian chipmunks with 20 seeds of *P. koraiensis* of each coating treatment at the center of each paved enclosure. The numbers of scatter-hoarded seeds of each treatment in the shallow pits were compared to see the influence of seed odor on scatter-hoarding of Siberian chipmunks.

Experiment 3

Effects of olfactory bulb ablation on cache pilferage and scatter-hoarding

Prior to olfactory bulb ablation, ten chipmunks (5♀, 5♂) actively participating in scatter-hoarding were selected and numbered. Following the same procedure as described in experiment 1, each of them was provided with 45 artificial caches each containing one seed of *P. koraiensis* in each shallow pit, allowing them to pilfer seeds freely in the paved enclosures. Pilferage rates were recorded 4 hours after establishment. Then, 60 seeds of *P. koraiensis* were given to each of the animals for scatter-hoarding freely in the semi-natural enclosures for eight hours. Scatter-hoarding intensity was recorded according to the number of seeds cached by Siberian chipmunks.

The olfactory bulb treatment has been shown to be effective at reducing olfactory capacity in other rodent species (Davidson et al. 2001). The olfactory bulb ablation of ten chipmunks was conducted in the present study. The experimental chipmunks were anesthetized and then firmly positioned to ensure stability during the operation. One small hole (2 mm in diameter, 12 mm anterior to bregma and 2 mm from the midline) were drilled by using a portable sterilized dental drill (diameter = 0.8 mm) and the two

olfactory bulbs were carefully smashed. Finally, 75% ethanol was administered to inhibit infection. Subjects were allowed to heal for 7 days following surgery. After healing, we repeated the above cache pilferage (in the paved enclosure) and scatter-hoarding (in the semi-natural enclosure) experiments using the olfactory bulbectomized chipmunks, to see if olfactory bulb ablation affected cache pilferage rate and scatter-hoarding intensity. To minimize the numbers of animals operated on, we did not conduct a control of a sham operation. Our results were also consistent with reduced olfactory ability assessed through other means.

Experiment 4

Relationship between olfactory ability and scatter-hoarding

Twenty animals (10♀, 10♂) were randomly selected and individually introduced in each of the semi-natural enclosures. Each animal was provided with 60 tagged *P. koraiensis* seeds to eat and scatter-hoard. Seeds were labeled using methods slightly modified from those reported in previous study (Yi et al. 2012): a white flexible plastic tag (2.5 × 3.5 cm, < 0.3 g) was attached to each seed using a 10-cm thin steel wire. Each animal was released into one semi-natural enclosure at 9:00 a.m. Seeds were placed at the center of each enclosure and were the only available food source. We checked seed fates at the end of each trial at 17:00 p.m. Seed fates were classified into six categories: 1) intact in situ (IIS), 2) eaten in situ (EIS); 3) eaten after removal (EAR); 4) intact after removal (IAR); 5) scatter-hoarded (SH), and 6) larder-hoarded (LH).

Eleven chipmunks (5♀, 6♂) that scatter-hoarded less than 10% (0–6.7%) of seeds released were regarded as non-hoarding animals (Pan et al. 2013). While, nine chipmunks (5♀, 4♂) that scatter-hoarded more than 20% (23.3–66.7%) of the seeds were considered as hoarding animals. Then, we randomly established 30 artificial caches in each of the semi-natural enclosures for the two distinct groups of animals. One seed was buried 2 cm deep in soil to create artificial caches. One animal was introduced in each enclosure at 9:00 a.m. and allowed to pilfer the artificial caches for four hours. After that, we checked how many artificial caches were excavated to evaluate chipmunk olfactory ability. Fates of artificial caches were classified into three types: not excavated and intact in situ (IIS), excavated and eaten in situ (EIS), and excavated and carried away to somewhere unknown (M).

Following behavioral experiments, all animals (including the ones who had got the bulbectomies smashed) were released at the original sites of capture after carefully checked to ensure that they were in good condition. All experiments were approved by the Ethical Committee for Animal Research of Jiangxi Normal University and the ethical treatment of animals was ensured throughout the trials.

Data analysis

We used Statistical Package for the Social Sciences (SPSS 16.0) for data analyses. One-way ANOVA (LSD, least significant difference) was used to see the difference in seed fresh mass and volatile compound emission among different seed soaking and coating treatments. We performed an ANOVA

using a general linear model (GLM) to test for differences in cache pilferage rates and scatter-hoarding intensities for seed soaking and coating experiments. Animal genders and seed treatments were regarded as fixed factors. The same method was used to see if there was difference in cache pilferage rates and scatter-hoarding intensities of Siberian chipmunks after olfactory bulb ablation, with animal genders and animal treatments as fixed factors. We also used GLM to test for intraspecific difference in olfactory abilities of chipmunks with different scatter-hoarding abilities. All proportional data was arc-sine transformed before analyses.

Data deposition

Results

Experiment 1 and 2

Effects of seed odor on cache pilferage and scatter-hoarding
Water soaking treatment significantly changed seed mass of *Pinus koraiensis* (F = 5.018, DF = 2, p = 0.014). Although four hours' soaking increased seed mass compared to the control (p = 0.004), the difference seems to be negligible (0.035 g). Ion abundance analyses showed that seed soaking significantly increased emission of α-pinene (F = 25.541, DF = 2, p = 0.001) and isopropyl toluene: F = 2.646, DF = 2, p = 0.002), but decreased evolution of camphene

(F = 7.511, DF = 2, p = 0.023) (Fig. 1A). Seed soaking neither significantly increased cache pilferage by Siberian chipmunks (F = 1.008, DF = 2, p = 0.377) nor decreased the amount of scatter-hoarding (F = 3.879, Df = 1, p = 0.479). Males scatter-hoarded more (F = 7.064, DF = 1, p = 0.016), but pilfer less seeds (F = 3.879, DF = 1, p = 0.058) than females.

Membrane-coating significantly decreased emission of odorous compounds from seeds of *P. koraiensis* based on the measurements of ion abundances, with the wholly-coated seeds emitting extremely low seed odor signals compared to the control (α-pinene: F = 86.039, DF = 2, p = 0.001; camphene: F = 14.425, DF = 2, p = 0.005; isopropyl toluene: F = 13.231, DF = 2, p = 0.006) (Fig. 1B). Post hoc tests showed that the uncoated seeds emitted more α-pinene than the partially- and wholly-coated seeds (all p < 0.05), while no significant difference was detected between the partially- and wholly-coated seeds (p > 0.05). The same patterns were observed for camphene. Differently, the partially-coated seeds emitted less isopropyl toluene than the uncoated and wholly-coated seeds (all p < 0.05), while the latter two treatments showed no difference (p > 0.05). Compared with the non-coated seeds of *P. koraiensis*, wholly-coated seeds were less pilfered by Siberian chipmunks (F = 6.500, DF = 2, p = 0.003) (Fig. 2A). More wholly-coated seeds were marginally scatter-hoarded by Siberian chipmunks (F = 3.434, DF = 2, p = 0.051) (Fig. 2B). There was no significant difference between males and females in pilfering seeds in artificial caches (F = 2.086, DF = 1, p = 0.156). But males scatter-hoarded more seeds than females (F = 11.152, DF = 1, p = 0.003).
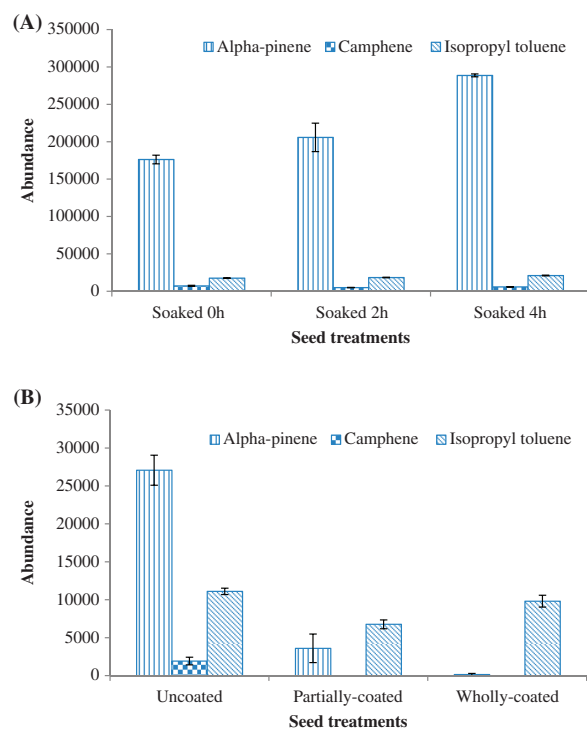

Figure 1. Volatile compounds emitted from the soaked and coated seeds of *P. koraiensis*. (A) soaking treatment (all n = 3); (B) coating treatment (all n = 3). Data are expressed as mean ± SD.
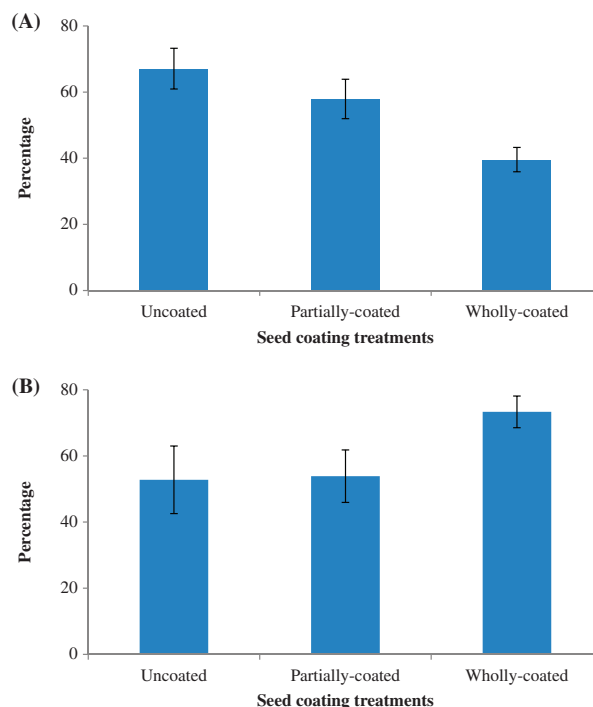

Figure 2. Effects of seed coating on cache pilferage (n = 16) and seed scatter-hoarding (n = 9) of Siberian chipmunks. (A) cache pilferage; (B) scatter-hoarding. Data are expressed as mean percent ± SE.

Experiment 3

Effects of olfactory bulb ablation on cache pilferage and scatter-hoarding

Olfactory bulb ablation significantly reduced the artificial cache pilferage rate by Siberian chipmunks (F = 33.894, DF = 1, p < 0.001; Fig. 3A), but increased the proportion of sham caches excavated by the chipmunks (F = 157.801, DF = 1, p = 0.001) (Fig. 3A), and the proportion of scatter-hoarded seeds (F = 206.688, DF = 1, p < 0.001) (Fig. 3B). Females pilfered more artificial caches but scatter-hoard less seeds than males (F = 5.528, DF = 1, p = 0.032; F = 13.936, DF = 1, p = 0.002). No significant difference was found in the proportion of excavated empty caches between males and females (F = 0.062, DF = 1, p = 0.806).

Experiment 4

Effects of olfactory ability on scatter-hoarding

There were significant differences in the proportions of scatter-hoarded seeds between hoarding and non-hoarding groups of chipmunks in the semi-natural enclosures (F = 93.925, DF = 1, p < 0.001) (Fig. 4A). Hoarding chipmunks pilfered fewer artificial caches than non-hoarding chipmunks (F = 8.137, DF = 1, p = 0.011) (Fig. 4B). Moreover, seeds excavated from artificial caches were more likely to be eaten in situ by non-hoarding chipmunks than by hoarding animals (F = 15.924, DF = 1, p = 0.001) (Fig. 4B).

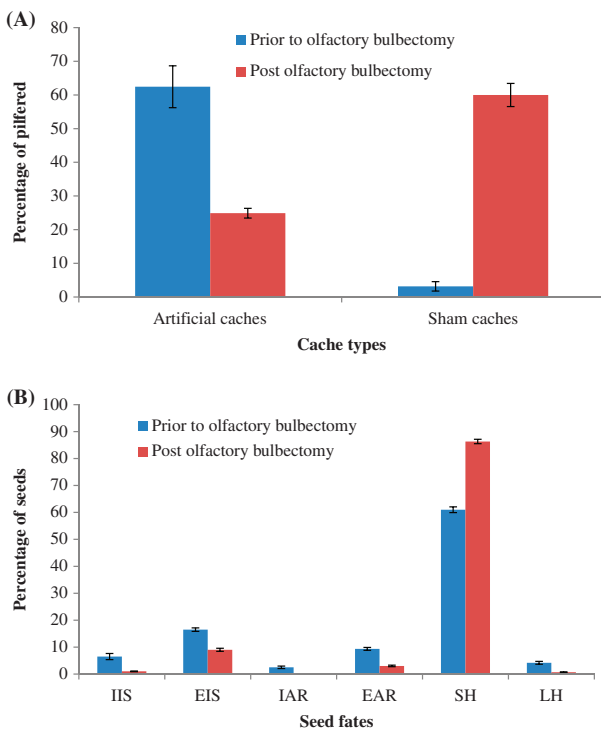Figure 3. Effects of olfactory bulb ablation on cache pilferage and seed scatter-hoarding of Siberian chipmunks. (A) cache pilferage (n = 10); (B) seed scatter-hoarding (n = 10). IIS, EIS, EAR, IAR, SH and LH refer to seeds intact in situ, eaten in situ, eaten after removal, intact after removal, scatter-hoarded and larder-hoarded, respectively. Data are expressed as mean ± SE.
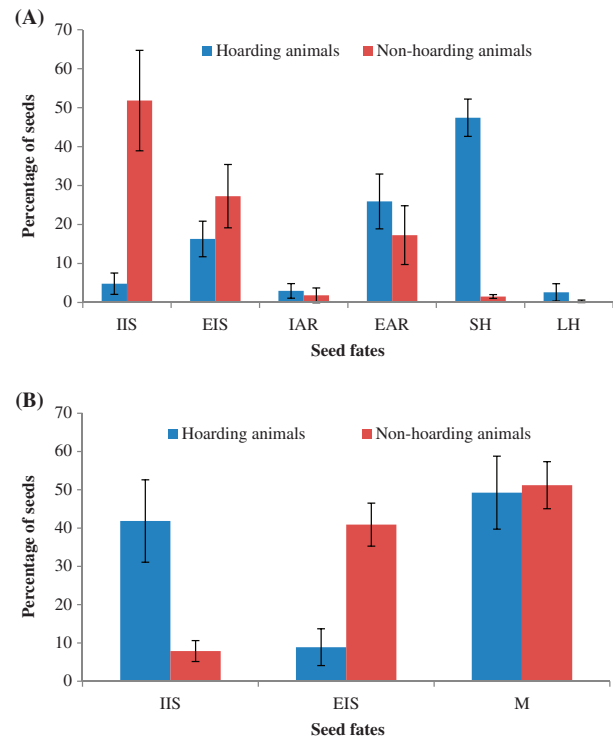

Figure 4. Relationship between the amount of scatter-hoarding and cache pilferage ability of hoarding (n = 9) and non-hoarding (n = 11) Siberian chipmunks. (A) the amount of scatter-hoarding; (B) pilferage ability. IIS, EIS, EAR, IAR, SH, LH refer to seeds intact in situ, eaten in situ, eaten after removal, intact after removal, scatter-hoarded, and larder-hoarded, respectively. M stands for seeds taken by the chipmunks from a cache site. Data are expressed as mean ± SE.

Discussion

The mechanisms by which scatter-hoarding by animals evolves have long been debated (Clarke and Kramer 1994). Cache pilferage and pilferage avoidance are likely key factors in affecting hoarding patterns of animals (Vander Wall and Jenkins 2003, Dally et al. 2006, Leaver et al. 2007, Rusch et al. 2013, Steele et al. 2014). Although olfaction cues have been reported to be closely linked to cache pilferage (Vander Wall 1990, Briggs and Vander Wall 2004, Taraborelli et al. 2009, Hollander et al. 2012, Paulsen et al. 2013), their effects on scatter-hoarding patterns have not ever been tested. Here, we showed that seed odor and olfactory ability of animals significantly affected seed scatter-hoarding intensity of animals, which is well connected to changes of their ability of pilferage. Our results support the hypothesis stating that weak olfaction cues between seeds and scatter-hoarding animals manipulate seed hoarding patterns of animals. Our study suggests that olfaction likely plays a key role in the evolution of both seed odor and scatter-hoarding behavior of animals, and may have significant effects on the interactions between seeds and scatter-hoarding animals. Although we have no knowledge whether male and female individuals exhibit different olfactory abilities, male chipmunks appear to show cache recovery advantage over female ones, mainly based on spatial memory (Pan et al. 2013). This may

partially explain why male chipmunks scatter-hoarded more seeds than female ones in our study.

The odor signal that seeds emit is especially important to animals that collect seeds on the ground or steal seeds from other animals' caches (Vander Wall and Jenkins 2003, Paulsen et al. 2013). Seed odor emission has been shown to affect cache pilferage and recovery (Vander Wall 1998, 2000, Jorgensen 2001). However, how seed odor mediates scatter-hoarding patterns of animals had not previously been explored. Our study suggests that food-hoarding rodents seem to prefer seeds with a weak odor for scatter-hoarding. Weak olfaction cue between seeds and seed-eating animals is expected to not only benefit cache rewards to food-hoarding animals but also seed survival after dispersal. Our results also support the observations that seeds emitting strong odors are easily detected and more likely to be pilfered, whereas seeds that produce weak odors are less likely to be pilfered (Hollander et al. 2012, Paulsen et al. 2013), showing the role of seed odor in manipulating cache pilferage by hoarding animals. Although soaking did not significantly increase cache pilferage by Siberian chipmunks, it is implausible that seeds in artificial caches are conspicuous to foragers because they were buried 2 cm deep in the fine sand. Some may argue that the water treatment will be immediately altered by saliva when chipmunks carry seeds in their mouths. However, seed mass was not changed by soaking for two hours, which is much longer than the handling time in mouth when caching by chipmunks ($<$ 2 min, XY unpubl.). Therefore, the possible explanation could be that the high ventilation capability of sand in pits, which facilitates seed detection by Siberian chipmunks, outweighs the differences in odor intensities of *Pinus koraiensis* seeds soaked for different times. Nevertheless, caching seeds with weak odor signals appears to reduce cache pilferage by pilferers, but may not influence cache recovery by the hoarders, because spatial memory for the locations of caches is the principal mechanism of cache recovery by scatter-hoarding animals (Vander Wall et al. 2008). Thus, our study suggests that seed odor signals not only affect pilferage, but may also influence scatter-hoarding intensity of animals. Scatter-hoarding behavior by food-hoarding animals in response to seeds with different odor signals is consistent with there being natural selection on seeds to produce weak odors (Hollander et al. 2012), possibly explaining why some plant species produce seeds with hard coat or deep physical dormancy to reduce their odor emission (Paulsen et al. 2013, 2014). Although many species of plant seeds mainly dispersed by rodents emit a variety of odors, several rodent species have evolved the behaviors of hulling, defleshing, or saliva-clumping when caching seeds to reduce cache pilferage by decreasing olfactory cues (Jansen et al. 2010, Yi et al. 2012, Jenkins and Devenport 2014).

Our study shows that olfactory bulb ablation reduces olfactory ability of food-hoarding animals, as indicated by the decrease in artificial cache pilferage ability. Siberian chipmunks with decreased olfactory ability tend to search for seeds in artificial caches mainly through exploratory digging, as more sham caches were excavated after olfactory bulb ablation. These results confirm that olfaction is of importance in affecting cache pilferage in food-hoarding animals (Vander Wall 2000). Siberian chipmunks appear to scatter-hoard more seeds after olfactory bulb ablation.

Moreover, Siberian chipmunks with lower olfactory ability to pilfer artificial caches show higher scatter-hoarding intensity, and vice versa, indicating that olfactory cues between seeds and scatter-hoarders could mediate scatter-hoarding behavior of animals. Since reciprocal pilferage is common in rich communities of scatter-hoarding animals (Vander Wall and Jenkins 2003); decrease in olfaction will lead to decreased ability of food-hoarding animals to pilfer food from other animals. Siberian chipmunks might prefer to cache more seeds that are less odoriferous because they may not be detected easily by pilferers. Our study suggests that olfaction plays an important role in shaping scatter-hoarding intensity of food-hoarding animals, and that there might be a tradeoff between scatter-hoarding intensity and olfactory ability at least at intraspecific level of food-hoarding animals. It is notable that our experiment of olfactory bulb ablation was lacking of sham-operated group, which needs to be considered in future studies.

Our study also implies that seed odor signals should be taken into account when exploring the effects of seed traits on food-hoarding behavior of animals, and then interactions between seeds and animals. For instance, physical and nutritional characteristics are unable to explain well the patterns of seed scatter-hoarding by animals (Lai et al. 2014, Wang et al. 2014, Zhang et al. 2016). Thus factors influencing the mutualistic connections between plants and animals by scatter-hoarding behaviors are still unclear (Wang et al. 2013). Preference for seeds with different traits and the mutualistic network between plants and animals might be understood better by considering seed odor properties. Moreover, our study supports the role of olfaction in shaping the evolution of scatter-hoarding of animals, as well as mutualistic/predatory interactions among multiple plant–animal species. Given that olfactory ability differentiates at intra- and inter-specific levels (Leaver and Daly 2001, Briggs and Vander Wall 2004, Vander Wall et al. 2009), difference in scatter-hoarding ability can also be expected. Thus, a tradeoff between olfactory ability and scatter-hoarding intensity is needed to be investigated at intra- and inter-specific levels in future studies.

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