Effects of frugivorous birds on seed retention time and germination in Xishuangbanna, southwest China

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ABSTRACT
The dispersal of many plants depends on transportation by birds as seed dispersers. The birds play an important role in long distance seed dispersal and may also affect seed germination. However, for plants who have many bird dispersers, the influence of dominant and non-dominant dispersers on retention time (dispersal distance) and germination remains poorly understood. In this study, we performed experiments with captive frugivorous birds and fruiting plant species to study the effects of dominant and non-dominant dispersers on seed retention time (SRT) and germination (seed germination percentage and germination speed). Our study showed a great interspecific variation in the effects of frugivorous birds on both SRT and germination. Some birds enhance the germination of a given plant species, but others do not. Generally, the dominant visitors improved the seed germination and performed longer seed retention time.

Keywords: Dominant visitors; Frugivores; Interaction; Seed germination; Xishuangbanna

INTRODUCTION
Seed dispersal by frugivores is a critical ecosystem process found throughout the world (Jordano, 2000; Schleuninger et al, 2011). Benefits of seed dispersal include avoiding inbreeding depression as well as avoiding a high density of predators and pathogens near their parent trees (Janzen-Connell hypothesis) (Bell et al, 2006; Connell, 1971; Janzen, 1970; Mangan et al, 2010; Swamy et al, 2011). Seed retention time (SRT), the time elapsed from fruit ingestion to seed elimination/defecation (Fukui, 2003), dictates the effective seed dispersal distance (Wotton et al, 2008). Some researchers have shown that SRT usually varies among different plant species due to differences in seed size (Chang et al, 2012; Figuerola et al, 2010; Fukui, 2003). There is also a positive correlation between SRT and the body size of the frugivores (Murphy et al, 1993; Spiegel & Nathan, 2007).

However, comparing SRT between different bird species has been rarely studied, and existing studies have produced inconsistent results. For instance, Spiegel & Nathan (2007) reported that the SRT of *Ocrbadenus baccatus* consumed by Tristram's grackle (*Onychognathus tristrami*) was much longer than those seeds consumed by Yellow-vented bulbul (*Pycnonotus xantbopygos*). On the other hand, Figuerola et al (2010) showed no differences between the SRT for the plants *Scirpus littoralis* and *S. maritimus* ingested by four waterfowl species.

Apart from avoiding potential Janzen-Connell effects, frugivores can also change seed germination success through the digestive processing of the seed. Many studies have shown that the digestive systems of frugivores significantly influence seed germination (Robertson et al, 2006; Samuels & Levey, 2005; Travest & Verdú, 2002). For example, digestion of frugivores can either increase (Reid & Armet, 2011) or decrease (Lieberman & Lieberman, 1986) the percent of seed germinated or the germination speed (and can reduce predator detection and consumption of seeds as well as attacks by pathogens) (Fricke et al, 2013). The effects of gut passage on seed germination usually differs between pairs of bird and plant species (Barea et al, 1991; Travest et al, 2001b). Frugivorous birds are considered to be important seed dispersers in ecosystem processes (Shanahan et al, 2001). The interaction between frugivorous birds and fruiting plants has been examined in a range of studies (Jordano, 1995). But the relationship between frugivorous birds and fruiting plants has often been asymmetrical (Mccann et al, 1998; Paine, 1992) and not all frugivores are effective dispersers to the plant species consumed (Bradford & Westcott, 2011). For example, for a given species of plant, not all the visiting birds but only one or two bird species had a mutualistic and compact relationship.

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with it (Silveira et al, 2012). As such, in our study, we classified birds into “dominant” and “non-dominant” visitors according to their observed visiting frequency to seven plant species. Then we compared the different effects between the two groups of birds on SRT and seed germination for these plant species.

MATERIALS AND METHODS

Study areas and species
This study was conducted in Xishuangbanna Tropical Botanical Garden (570 m a.s.l., centered at N21°55′, E101°16′), in Yunnan Province, southwest China. Our study area lies within the Indo-Burma biodiversity hotspot and contains a high diversity of fruiting plants within the same day (Fukui, 2003).

Six common frugivorous bird species were selected for the experiment: Red-whiskered Bulbul (Pycnonotus jocosus), Sooty-headed Bulbul (Pycnonotus aurigaster), Black-crested Bulbul (Pycnonotus melanicterus), Blue-throated Barbet (Megalaima asiatica), Plain Flowerpecker (Dicaeum concolor), and Crested Myna (Acridotheres cristatellus). These species were selected because they are the most common and abundant species in our study site, and consume large amounts of fruits from a wide variety of plant species (Yang, 1994, 2004). All individuals were kept in separate cages (30 cm×30 cm×40 cm, length×width×height) and fed daily diets consisting of apple, pear, bananas, and mealworms. Water was supplied ad libitum throughout the experiment. All Plain Flowerpecker individuals were released at the beginning of the experiment because they did not consume any fruits in captivity.

Field observation
Field observations were conducted from July 2012 to July 2013, during the fruiting period of the seven target species. Three trees or clusters for each plant species were selected to record the number of individuals of each bird species that visiting the plant species; field observation was carried out at peak times of avian activity (0700-0830h, 1600-1730h). During the observation, the entire tree or cluster of plants were scanned once every 10 minutes with a 10×42 binocular (Olympus). Each tree or cluster was observed for 3 days in total with 3 hours in each day. We defined the dominant visitors as the species with the largest number of individuals during the observation period.

Seed retention time
Thirty-three individuals of birds were used in the SRT experiment, including 8 Red-whiskered Bulbuls, 6 Sooty-headed Bulbuls, 8 Black-crested Bulbuls, 5 Blue-throated Barbets and 6 Crested Mynas, respectively. To ensure that birds would consume fruit from the start of the trial onwards, and thereby standardize the SRT, the maintenance diet was removed the night before trials (Tewksbury et al, 2008). The experiments began in the morning (0800h) and the birds were given 30 fruits of a single plant species. The fruits were subsequently removed 10 minutes after the first fruit was eaten and a moveable plastic tray was placed at the bottom of the cage to collect the seeds defecated every 5 minutes (Spiegel & Nathan, 2007). The experiment ended when no seed was observed in the faeces for 60 minutes. We used the midpoint of the first 10 free feeding minutes (5 minutes after the first fruit was eaten) as the beginning of the test. The mean SRT for each fruit species was defined as the mean retention time for all defecated seeds from a single bird.

Seed germination
Twenty fruits of each plant species were given to each bird. In total, 7 plant species, 4-5 bird species, and 5 individuals for each bird species were used for this experiment. After all of the seeds of the 20 fruits were excrated, they were extracted from the faeces and dried at room temperature. Some individuals of Crested Myna appeared ill during the experiment, and they were released before the experiment ended. Therefore, for the myna we have consumption and germination trials for 3 plant species (Myers et al, 2000). Four-hundred-thirty-four bird species have been recorded in this region, constituting 36.3% of the avian richness in China (Jiang et al, 1998).

To better understand the effect of dominant birds and non-dominant birds on fruiting plants, we selected seven common fleshy fruited species that are visited by birds and non-avian frugivores (Table 1). The seeds of one of the species, Ophiopogon bodinieri, were divided into two types: O. bodinieri (big) and O. bodinieri (small), as their fruits were significantly different in size (mean±SD, 9.19±0.55 mm vs 8.19±0.47 mm, t=7.608, P<0.001). To minimize the intraspecific variation in fruit features, fruits were collected from either a single mother tree for woody plants or a cluster of individuals for herbaceous plant species.
were computed are as follows: percentage (GP) and germination speed (GS). The indices various bird species and control seeds): final germination when no seeds germinated for 2 consecutive months. These (Traveset et al, 2001a). Seed germination checks would stop non-germinated seeds (Mandon-Dalger et al, 2004). Seeds were considered germinated when the radicle emerged (Traveset et al, 2001a). Seed germination checks would stop when no seeds germinated for 2 consecutive months. These non-germinated seeds were all uninvolved due to fungus infection or rot. Two indices were introduced to estimate the germination differences between the different treatments (digested by various bird species and control seeds): final germination percentage (GP) and germination speed (GS). The indices were computed as follows:

\[ GP = \frac{N_1 + N_2 + \ldots + N_n}{20} \times 100\% \] (1)

\[ GS = \frac{N_1}{1 + N_2 + \ldots + N_n} \] (2)

Where, \( N_n \) expresses the number of germinated seeds in day \( n \).

Data analysis

A one-way ANOVA with Tukey's honest significant difference (HSD) multiple comparisons was used to analyze the differences of GP, GS and mean SRT among various bird species for each plant species. A square root transformation was applied to make the mean SRT values exhibit homoscedasticity and normality. All analyses were performed in R 3.1.1 (The R Core Team, 2014).

RESULTS

Visiting frequency of birds to each plant species

There were 27 hours of observation for each plant species and 216 (because \( O. bodinieri \) were divided into two types) hours in all. Eleven species and 1,021 individuals of bird were observed in total. No birds visited \( O. bodinieri \) (small and big) and \( A. squamulosa \). The Red-whiskered Bulbul was the dominant visitor of \( L. glutinosa \), \( S. hainanense \), \( P. suberosa \) and \( M. paniculata \) with visiting frequencies of 90.34\%, 43.33\%, 45.61\% and 79.17\%, respectively (Figure 1). The Plain Flowerpecker was the dominant visiting species for \( D. pentandra \) (75.25\%) (Figure 1).

Seed retention time

Overall, 181 experiments of 7 plant species were conducted; this was less than anticipated because not all the plant seeds were eaten within the regulation time (10 minutes) by some of the bird species (Appendix 1). The mean SRTs varied significantly between bird species for the plants \( P. suberosa \) (Figure 2A), \( M. paniculata \) (Figure 2B), \( D. pentandra \) (Figure 2C) and \( O. bodinieri \) (small) (Figure 2D). For \( P. suberosa \), the mean SRTs varied significantly among treatments for both \( P. suberosa \) (Figure 2A), \( M. paniculata \) (Figure 2B), \( D. pentandra \) (Figure 2C) and \( O. bodinieri \) (small) (Figure 2D). For \( P. suberosa \), the mean SRTs varied significantly between bird species (Figure 2D).

Seed germination

No seeds of \( L. glutinosa \) and \( M. paniculata \) germinated from any treatment. The GP significantly differed among treatments for both \( P. suberosa \) (Figure 3A), \( M. paniculata \) (Figure 3B), \( D. pentandra \) (Figure 3C), \( O. bodinieri \) (big) (Figure 3D), \( O. bodinieri \) (small) (Figure 3E) and \( A. squamulosa \) (Figure 3F). Seeds of \( P. suberosa \) and \( M. paniculata \) digested by their dominant visitors showed a longer mean SRT (Figure 3C, D).

The frequency of different visitors to each plant species

Figure 1 Frequency of different visitors to each plant species

![Figure 1](image-url)
Figure 2  Seed retention time of the six plant species
A: L. glutinosa; B: S. hainanense; C: P. suberosa; D: M. paniculata; E: O. bodinieri (big); F: O. bodinieri (small); G: A. squamulosa. Different lowercase letters indicate a significant difference (P≤0.05); Black bars represent the dominant visitor for the given plant species.

Figure 3  Germination percentage of digested and control seeds of the five plant species
A: S. hainanense; B: P. suberosa; C: D. pentandra; D: O. bodinieri (big); E: O. bodinieri (small); F: A. squamulosa; Different lowercase letters indicate a significant difference (P≤0.05); Black bars represent the dominant visitor for the given plant species.
DISCUSSION

Previous studies have usually chosen one or a few species of birds that consume a particular plant species and have then compared differences between these bird species on SRT and germination (Barnea et al., 1991; Charalambidou et al., 2003; Figuerola et al., 2010; Fukui, 2003; Jordaan et al., 2011). However, the effects of birds on plants varies between both bird and plant species (Traveset & Willson, 1997; Yagihashi et al., 1999). As such, our study considered a range of avian frugivore and plant species pairs.

In general, the process of seed passage through the avian digestive tract is a key aspect of endozoochory, and the SRT is a significant factor for both plants and birds (Fukui, 2003). Our results showed that SRT exhibited significant variation among avian frugivores visiting four plant species (P. suberosa, M. paniculata, O. bodinieri (big), O. bodinieri (small)), similar to previous studies of Jordaan et al. (2011), but inconsistent with the result of Figuerola et al. (2010). The variation of SRT may be due to differences in disperser body size (Spiegel & Nathan, 2007), seed size consumed (Figuerola et al., 2010; Fukui, 2003), or some combination thereof. Some studies have suggested that seed dispersal distance is a function of SRT (Fukui, 2003). Thus, the variation of SRT among bird species may lead to wide variation in seed dispersal distance.

Once ingested by birds, seeds may experience both chemical and mechanical processing in the digestive system. As a result, the seed coats or endocarp may be altered, thereby affecting germination (Barnea et al., 1990). However, whether digestion increases seed germination is unclear (Traveset, 1998). In our study, digestion significantly affected both GP and GS for some species but not for others. For plants which had bird visitors in the field (S. hainanense, P. suberosa, D. pentandra), both GP and GS were improved through bird digestion. For the three species which had no bird visitors in the field (O. bodinieri (big), O. bodinieri (small), A. squamulosa), the GP and GS were not influenced by birds ingesting. For the plant species that have bird consumers, it looks that birds are not only seed dispersers but also germination enhancers, suggesting that their relationship is a mutualism.

By dividing the visitors into dominant and non-dominant ones, we found that the SRT of a given plant species digested by
dominant visitors was either the same as or longer than that consumed by non-dominant visitors. The GP and GR for *P. suberosa* and *D. pentandra* were much higher after consumption by the dominant visitors than by some non-dominant visitors as well as control ones. Therefore, compared to non-dominant visitors, the action on seed retention time and germination of dominant visitors is more prominent. Perhaps the different effects on seed germination and retention time by dominant and non-dominant visitors can also help to explain why some previously studies reported inconsistent results.

In conclusion, this study supports findings in the literature that frugivorous birds have positive effects on seed retention time and ultimately seed germination. Moreover, dominant visitors seem to confer more benefits than non-dominant visitors to a plant species, suggesting that future studies should consider the visiting frequency of frugivorous birds as an important ecological factor when studying gut passage time of seeds and how birds affect seed germination.

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**Appendix 1 The effective sample size of SRT experiment**

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Blue-throated Barbet</th>
<th>Red-whiskered Bulbul</th>
<th>Sooty-headed Bulbul</th>
<th>Black-crested Bulbul</th>
<th>Crested Myna</th>
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<td><em>L. glutinosa</em></td>
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<td>6</td>
<td>8</td>
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<tr>
<td><em>S. hainanense</em></td>
<td>5</td>
<td>5</td>
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<tr>
<td><em>P. suberosa</em></td>
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<td>5</td>
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<tr>
<td><em>M. paniculata</em></td>
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<tr>
<td><em>O. bodinieri</em> (big)</td>
<td>5</td>
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<tr>
<td><em>O.bodinieri</em> (small)</td>
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<tr>
<td><em>A. squamulosa</em></td>
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