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Germination response of *Lithraea molleoides* seeds is similar after passage through the guts of several avian and a single mammalian disperser

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Abstract

Seed dispersal by vertebrate frugivores plays an important role in plant population dynamics and community structure. The gut treatment may modify the germination response of seeds; often the specific effects of seed ingestion are not consistent among frugivorous taxa. In the Chaco mountain woodlands of Argentina, an ecosystem threatened by human activities, frugivorous birds enhance the seed germination of the most abundant fleshy-fruited plants. However, the effect of the identity of dispersers on seed germination remains unknown. In this work, we evaluated and compared the seed germination response of *Lithraea molleoides* (the dominant tree of the region) to gut passage through three *Turdus* species and the Pampa Fox (*Lycalopex gimnocercus*). Due to anatomical differences between *Turdus* species and Pampa Fox, we expected to observe higher seed germination in the seeds treated by the gut of *Turdus* species compared to those that have passed through the Pampa Fox’s gut. Our results showed that germination response of *L. molleoides* seeds was positively related to gut passage through *Turdus* species and Pampa Foxes (without differences among seed dispersers). Consequently, both the avian species and the Pampa Fox contribute positively to the dispersal and germination of *L. molleoides* seeds.

**Keywords:** Chaco Mountain Woodland, endozoochory, Pampa fox, seed germination, *Turdus* species.
Introduction

Seed dispersal by vertebrate frugivores plays an important role in the dynamics of some plant populations and communities (Jordano and Herrera 1995; Ness et al. 2006). This form of dispersal is considered crucial in the life cycle of some fleshy-fruited plants, allowing offspring to colonize surrounding safe sites (Wenny 2001) and/or sites far from the parent plants (Janzen 1970). This can increase the chances of seed’s survival and later adult establishment (Howe and Smallwood 1982). Therefore, assessments of the role of vertebrate frugivores on seed dispersal are essential to understanding the processes of maintenance and regeneration of those plant communities (Traveset 1998; Traveset et al. 2007). The effect of vertebrate fruit ingestion on seed germination is an aspect of the seed dispersal process that has received considerable attention in the literature (Traveset and Verdú 2002; Schupp et al. 2010; Traveset et al. 2007; Duron et al. 2017). Seed dispersal of endozoochorous plants entails the ingestion of edible and nutritious fruits by animals and the later defecation or regurgitation of undamaged seeds (Howe 1986; Caziani and Protomastro 1994; Varela and Bucher 2002; Ponce et al. 2012; Oleksy et al. 2017).

A meta-analysis performed by Traveset and Verdú (2002) confirmed that the passage of seeds through the digestive tracts of vertebrate frugivores generally modifies the germination response of the seeds. However, they showed that the specific effects of seed ingestion are not consistent among frugivorous taxa, since in some cases germination may increase, decrease, or remain unchanged after gut passage. Generally, birds and bats had a greater positive effect on germination than either non-flying mammals or reptiles (Traveset and Verdú 2002). This pattern has been commonly attributed to differences in seed retention times during gut passage, which are positively associated with frugivore body size (Karasov and Levey 1990).
Studies of seed dispersal by frugivores in South American tropical forests have received more attention than other environments (e.g. Tabarelli and Peres 2002; Donatti et al. 2011; Blendinger et al. 2011). In the Chaco region, the biggest subtropical forest, there is a limited knowledge about seed dispersal by animals. Some studies from central Chaco (Varela and Bucher 2002; Varela et al. 2008) have shown a positive effect of reptile and mammal gut treatment on seed germination response for several Chacoan native plants (i.e. the qualitative component of seed dispersal effectiveness; e.g. Varela and Bucher 2002). Recently, a positive effect of avian gut treatment on seed germination response of the main fleshy-fruited plants of the Chaco mountain woodlands was found in Argentina (Díaz Vélez et al. 2017). Frugivorous bird species not only moved seeds to different microsites, but also provided different treatments to the seeds, enhancing the chances of seed germination and plant establishment in the Chaco mountain woodlands. However, the effect of the identity of disperser birds on seed germination or seed dispersal remains unknown.

The Chaco mountain woodlands in Central Argentina and, especially their fleshy-fruited plant species that are consumed and dispersed by vertebrates, are currently threatened by several human activities (e.g. fire, deforestation, urbanization and plant invasion; Gavier-Pizarro and Bucher 2004; Argañaraz et al. 2015; Giorgis et al. 2017). The dominant tree of this threatened region is Lithraea molleoides (Vell.) Engl. (Anacardiaceae) (Giorgis et al. 2017), which produces a great amount of drupes (fruit size: 4-8mm of diameter; Carmello-Guerrero and Paoli 2005; Obs. pers.). It has been found that L. molleoides fruit ingestion by birds may enhance the seeds germination response, involving disinhibition and scarification mechanisms as well (Díaz Vélez et al. 2017). Studying the role of mutualists in seed dispersal effectiveness would provide the basic knowledge for the restoration of the endangered Chaco mountain ecosystems. However, the frugivore assemblage associated with L. molleoides has not been previously reported. We identified several species of animals as potential seed dispersers (see supplementary Table S1), including common birds and mammals [birds: Turdus spp. and mammal:
Pampa Fox (Lycalopex gymnocercus)]. Hence, the confirmation of the legitimate disperser capacity for these common animals and their plausible differential effect on seed germination remains unclear, and its consideration would be important for passive restoration (e.g. Guidetti et al. 2016).

The objectives of this study were to evaluate and compare the germination response of L. molleoides seeds to gut passage through common birds (three Turdus species) and the Pampa Fox. Due to anatomical differences (i.e. size and gut length) between Turdus species and the Pampa Fox, we expected higher seed germination in the seeds following gut treatment by Turdus species than those in Pampa Fox’s gut. In addition, given the similar size among the three considered Turdus species (see Collar 2003), we expected similar seed germination responses among them. We measured seed germination responses as the final germination percentage, and the rate of germination defined as the speed at which seeds germinated.

Materials and methods

Study area

We carried out the study in the western slopes of the Sierras Grandes (32° 54’ S, 64° 57’ W), between 1000–1200 m a.s.l., east of Los Hornillos, Córdoba Province, Argentina (see supplementary material for details about study site).

Fruit collection and seed obtained from the faeces of birds and foxes
During the end of 2012 summer, we collected fruits from 25 *L. molleoides* plants on the same day and at the same site to avoid any seed age or source effects on germination. Our study considered the collected faeces of the following bird species: Chiguanco Thrush (*Turdus chiguano*), Creamy-bellied Thrush (*Turdus amaurochalinus*), and Rufous-bellied Thrush (*Turdus rufiventris*). All of these bird species avidly consume *L. molleoides* fruits found in the Chaco mountain woodlands (supplementary Table S1) and they are common frugivores of the area (Vergara-Tabares 2017). We captured seven adult individuals (four males and three females) of each bird species using mist nets in the study site. We then kept each bird in a separate cage (60×60×60 cm) and maintained them on a diet consisting of nutritionally balanced food used for poultry birds, worms, and fruits (i.e. apple, pear, and banana). At the time of experimental seed gut passage, we gave the birds only the *L. molleoides* fruits (samples taken from the mixture of the fruit collection obtained from those 25 *L. molleoides* plants). Birds were kept from 10 April to 20 April, and fruits of *L. molleoides* were offered during the last four days. At the end of the experiment, birds were released in the same site where they were captured.

The Pampa Fox (*Lycalopex gymnocercus*) is one of the most common carnivores in the Chaco woodlands and savannas (Ojeda et al. 2012), but they also incorporate fruits in their diet (Varela et al. 2008). Our field observation indicated that the faeces of Pampa Foxes frequently contained seeds of *L. molleoides*. We searched for Pampa Fox faeces walking along the Los Hornillos ravine, specifically focusing on open areas near to the canopy of *L. molleoides* trees, and following the recommendations of rural inhabitants. After a couple of walks we collected ten fox faecal depositions from sites separated by at least 150 m. We manually separated the seeds from the bird and fox faeces, and then placed them in paper bags and stored them in dark and dry conditions during one week until the date of sowing.

**Germination experiment**
For the germination experiment (trials: gut passage seeds and manually extracted seeds), we randomly selected seeds obtained from the faeces of birds and foxes (gut passage treatment), and we also manually extracted and then randomly selected seeds from fruits (manually extracted treatment) that have been previously stored (one week). We tested seed germination response in a germination chamber programmed for a 12 h light/12 h dark photoperiod (38 µmol m\(^{-2}\) s\(^{-1}\) cool white fluorescent light tubes) at 25/15ºC, respectively. This temperature regime is optimal for germination of the different species in the study area (Funes et al. 2009). We used six replicates per treatment and each replicate consisted of 20 seeds placed on filter paper in 9-cm diameter Petri dishes (Bravo et al. 2014). To avoid fungal growth we used an antifungal solution with bencimidazol once a week. For 110 days, we moistened seeds with distilled water every other day, counting and removing germinated seeds. We considered the seed germinated when the emerged radicle measured at least 2 mm (ISTA 1996). After each count, the Petri dishes were randomly moved between chambers to avoid a chamber position effect.

Data analysis

We tested the effects of the gut passage (birds and Pampa Fox species) and manually extracted seeds treatments on final germination percentages using a binomial generalized linear model (GLM). We estimated the rate of germination using: a) modified Timson index of germination velocity and b) the mean time to germination (MTG; see Baskin and Baskin 2014). The highest Timson index and the lowest MTG values indicate the highest rates of germination, respectively. For the treatment comparisons of the rate of germination, we used a one-way ANOVA with further comparisons made using Fisher’s post-hoc tests. Data were analyzed using Infostat software (Di Rienzo et al. 2002) and the results were considered statistically significant when \( P < 0.05 \).
Results

After 110 days of the experiment, the maximum percentage of germination was ~ 45% from seeds obtained of Rufous-bellied Thrush and Pampa Fox faeces (43.33 ± 13.29 and 44.17 ± 12.81, respectively). Final germination percentages differed significantly between treatments ($F_{4,25} = 2.92; P = 0.041$; Fig. 1, A). Fisher’s post-hoc test showed that final germination was significantly higher in gut passage seeds than that of manually extracted ones. Overall, final germination did not differ significantly between any of the three bird species or the Pampa Fox (Fig. 1, A).

The values of the Timson index and MTG differed significantly among treatments ($F_{4,25} = 5.28; P = 0.003$ and $F_{4,25} = 2.82; P = 0.046$, respectively; Fig. 1, B, C). Fisher’s post-hoc tests showed that the Timson index was higher and MTG lower in gut passage treatments than that of manually extracted ones. No differences were found among frugivorous species for both rates of germination indices (see Fig. 1, B, C).

Discussion

Our study revealed that the germination response of *L. molleoides* seeds was positively related to gut passage through *Turdus* species and Pampa Foxes; the final germination and the rate of germination were higher in comparison to seeds in the manually extracted treatment (Fig 1 A, B, C). Therefore, it is plausible that Chiguancos Thrush, Rufous-bellied Thrush, Creamy-bellied Thrush, and the Pampa Fox play an important role as the *L. molleoides* seed dispersers in the Chaco mountain woodlands.
*L. molleoides* seeds showed similar germination responses to ingestion by three species of *Turdus* species and by the Pampa Fox. Many studies (reviewed in Traveset 1998) have supported the idea that frugivore body size, gut morphology and diet type determine the time of gut passage, which influences the probability of successful germination. We observed similar germination responses after seed ingestion by congeneric *Turdus* species; this may be due to similarities in body size and gut characteristics (Ricklefs 1996; Collar 2003). Contrary to our expectations, the effect of gut passage of the Pampa Fox did not differ from the birds even though the fox’s size and digestive system are much greater than those of the *Turdus* species (see Jordano 2000). This pattern may be due to similar seed retention times among the animals analyzed, or particular characteristics of *L. molleoides* seeds. For instance, the seeds of *L. molleoides* present a glandular endocarp (Carmello-Guerreiro and Paoli 2005) that could reduce deleterious effects of gastric acids found in the digestive system of frugivore dispersers. However, further studies are necessary to elucidate the cause of our germination response pattern, for example comparing the seed gut passage time of the *Turdus* species and the Pampa Fox.

One of the most important consequences of vertebrate seed dispersal is the transport of seeds away from maternal plant to potentially safe microhabitats, which may enhance chances of seedling survival and later adult establishment (Howe and Smallwood 1982; Howe and Miriti 2004). Another advantage is the increase of final germination after seed ingestion (Schupp 1993). Our results demonstrated that members of Turdidae are legitimate seed dispersers in the Chaco mountain woodlands, supporting other findings in temperate regions (e.g. Herrera 1984; Compton et al. 1996; Li et al. 2000). The *L. molleoides* seed disperser assemblage included at least 14 species (categorized as seed dispersers or pulp consumers; see supplementary Table S1) and the home range used by the species of the assemblage can vary; thus seed dispersal may occur in a wide range of habitat types. The *Turdus* species are distributed differently along an altitudinal gradient and vary in their use of habitat (Salvador et al. 2017; Vergara-Tabares unpublished data). For example, the Chiguano Thrush is found mainly
between approximately 800 and 2700 m.a.s.l, at the top of the Córdoba Highlands, whereas Creamy-bellied Thrush and Rufous-bellied Thrush are commonly distributed between 1500 and 1800 m.a.s.l. At a small spatial scale, our observations (unpublished data) suggest that the Rufous-bellied Thrush uses shady and humid sites near water streams. We also found that the Pampa Fox is another legitimate seed disperser, as shown by Varela and Bucher (2006) who worked in the Chaco lowland woodlands. Pampa Foxes may utilize open areas according to the territorial demarcation (i.e. feaces depositions) found in this type of habitat; this behaviour has also reported for other fox species (e.g. Jimenez et al. 1991).

Further research would help to establish the relationship between the habitat used by the different seed dispersers and the quality of microhabitats, a key factor for seedling establishment.

In the present study, we elucidated part of the qualitative subcomponent of seed dispersal effectiveness of the *L. molleoides* and also showed that the Pampa Fox and three species of *Turdus* are legitimate seed dispersers. Seed germination response was enhanced after gut passage of these animals, suggesting the occurrence of disinhibition and/or scarification processes (see Díaz-Vélez et al. 2017). The outstanding fact of our study suggested that the population dynamics of the *L. molleoides* may depend on Pampa Foxes and the three species of *Turdus*. Also, our findings highlighted the importance of these birds and the mammal on passive forest restoration via the activity of these legitimate seed dispersers. Nonetheless, a better understanding of the influence of the seed disperser assemblage on the *L. molleoides* population dynamics is needed. Therefore, further research should focus on the quantitative components of seed dispersal effectiveness (e.g. relative importance on dispersal services of each disperser) and also other qualitative aspects (e.g. sites of seed depositions effect on plant establishment) (Schupp et al. 2010).

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References


Figure caption

Figure 1. Box plots of parameters of *Lithraea molleoides* seed germination response. (A) Final germination percentage, and (B, C) rate of germination, measured as: (B) Timson Index, and (C) Mean time to germination. The boxes correspond to the treatments: gut passage seeds [i.e. ingested seeds by three *Turdus* species: Chiguanco Thrush (CT), Creamy-bellied Thrush (CBT), Rufous-bellied Thrush (RBT), and the Pampa Fox (PF)] and manually extracted seeds (Dep). In all cases different letters indicate significant differences ($P < 0.05$; ANOVA and Fisher’s *post-hoc* tests).
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