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Physiological dormancy broken by endozoochory: Austral parakeets (*Enicognathus ferrugineus*) as legitimate dispersers of calafate (*Berberis microphylla*) in the Patagonian Andes

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Abstract

Aims Seed dispersal by endozoochory is an important process in plant regeneration and the establishment of new populations. Seeds with dormancy may especially benefit after disperser gut passage. However, the ways in which gut passage affect the germination of plant species with physiological dormancy remain unclear. Here, we experimentally assessed the mutualistic interaction between the Austral parakeet (*Enicognathus ferrugineus*) as a disperser of calafate (*Berberis microphylla*), a thorny bush inhabiting the understory of the Austral temperate forests of South America with seeds that are characterized by deep physiological dormancy.

Methods Germination success and viability of calafate seeds obtained from faeces and from intact fruits were tested under four treatments: (i) digested seeds, (ii) digested seeds with faecal extract, (iii) intact seeds from fruit and (iv) intact seeds from fruit with pulp.

Important Findings About 65% of the Austral parakeet droppings contained calafate seeds. Viability of seeds did not differ between treatments. However, germination was significantly higher in digested seeds than in intact seeds from fruits, while no difference was found between faecal and pulp extracts. Neither faecal matter nor fruit pulp provided seeds with any ecological advantages derived from enhancing germinability, but did confer some disadvantage in germination time. Faecal matter is expected to be completely lacking around seeds after several months under snow before germinating in the following spring, given intense washing due to persistent rain and the spring thaw in the Patagonian Andes. The higher germinability along with faster germination of digested seeds supports the hypothesis of a legitimate mutualistic interaction between Austral parakeets and calafate. We hypothesized that the passage through the disperser digestive tract might break physiological dormancy as differences in germinability between ingested and non-ingested seeds. Our results highlight the relevant role of endozoochory in plant species with physiological dormancy living in highly seasonal environments.

Keywords: seed dispersal, Austral forest, mutualism, gut passage, viability, germination success, germinability

摘要：种子由动物内携传播是植物再生和新种群建立的重要过程。具有休眠的种子经过传播者肠道传代作用后可能特别受益。然而，肠道传代作用如何影响具有生理休眠植物的萌发尚不清楚。在本研究中，我们实验性地评估了南鹦哥(*Enicognathus ferrugineus*)作为小檗属植物(*Berberis microphylla*)传播者的互惠共生。*Berberis microphylla*是一种多刺的灌木，栖息在南美洲温带森林的下层，其种子具有深度生理休眠的特性。本研究通过四种处理：(1)种子消化处理，(2)粪便提取物消化处理，(3)果实种子完整处理和(4)包含果肉果实种子完整

处理，测定了从粪便和完整果实中提取的*B. microphylla*种子的发芽成功率和活力。研究结果表明，大约65%的南鹦哥的粪便中含有*B. microphylla*种子。不同处理的种子存活率无显著差异。然而，消化后的种子萌发率显著高于完整果实种子，而粪便和果肉提取物处理之间没有差异。粪便和果肉都没有为种子提供任何提高发芽能力的生态优势，但在萌发时间上确实造成了一些劣势。由于持续降雨和巴塔哥尼亚安第斯山脉春季融雪带来的严重冲刷，种子附近的粪便在雪下几个月后就会完全缺失。消化种子较高的发芽能力和较快的发芽速度验证了南鹦哥和*B. microphylla*之间具有合理互惠共生的假设。因为消化和非消化种子发芽能力的差异，我们假设通过传播者的肠道传代作用可能打破种子的生理休眠。我们的研究结果强调了在具有高度季节性的环境下，具有生理休眠的植物物种中与动物内携传播的相关作用。

关键词：种子传播，南部森林，互惠共生，肠道传代，活力，发芽成功，发芽能力

INTRODUCTION

Endozoochorous seed dispersal is a widespread mutualistic interaction among fleshy-fruited plants and multiple animal species, with important implications for plant population dynamics and community structure (Fenner and Thompson 2005; Schupp *et al.* 2010; Snell *et al.* 2019; Traveset *et al.* 2014). Viable seeds defecated by vertebrates generally germinate at a higher frequency and faster than intact seeds from fruits, although much variation has been shown depending on species-specific traits, such as seed size, permeability, coat thickness and dormancy (Soltani *et al.* 2018; Traveset and Verdú 2002). Thick, water-impermeable seed coats are common in plants with physical dormancy (Baskin *et al.* 2000). This ensures the maintenance of viability and even enhances germination after disperser gut passage due to scarification of the seed coat (Jaganathan *et al.* 2016). Conversely, seed germination can decrease after gut passage in species with water-permeable seed coats (Soltani *et al.* 2018). These species can show various adaptations to enhance seedling establishment and survival, including morphological, physiological or morpho-physiological dormancy (Baskin and Baskin 2014). However, the ways in which gut passage affect seed germination in plants with these types of dormancy remain unclear.

Seeds with physiological dormancy show physiological inhibiting mechanisms in the embryo that prevent germination (Baskin and Baskin 2004), as an evolutionary adaptation to avoid germination during unsuitable ecological conditions (Finch-Savage and Leubner-Metzger 2006). These physiological mechanisms are generally triggered by some environmental factor breaking this dormancy, including temperature, light, darkness, or inorganic or organic chemicals (Baskin and Baskin 2014). The effect of gut passage could go beyond the physical effects on the seed coat in species with physiological dormancy, because seed scarification is not needed for germination (Baskin and Baskin 2014). For instance, germination after gut passage can be stimulated differently in species with physiological or physical dormancy, e.g. *Polygonum lapathifolium* or *Echinochloa crus-galli* (Soltani *et al.* 2018). While gut acids can help to break physical dormancy by digesting pulp containing germination inhibitors (Samuels and Levey 2005), these acids can also trigger several physiological processes directly acting on seed embryos to enhance germination in species with physiological dormancy. Whereas physiological dormancy can enhance seedling survival by delaying seed germination until favourable conditions arise, especially in seasonal environments (Fenner and Thompson 2005), there is still scarce knowledge on disperser species and on the factors linking gut passage with environmental conditions governing fruiting, seed dispersal and germination in plants with this kind of dormancy.

The general view that parrots (Psittaciformes) are seed predators has recently been challenged by studies demonstrating their crucial role as seed dispersers of many plants (Blanco *et al.* 2018; Tella *et al.* 2015). This paradigm shift has provided new fruitful research avenues on the processes governing plant communities and ecosystem functioning in the tropics (Baños-Villalba *et al.* 2017; Blanco *et al.* 2015, 2019; Montesinos-Navarro *et al.* 2017; Sebastián-González *et al.* 2019). The occurrence and viability of seeds dispersed by endozoochory have also been recently demonstrated for several parrot and plant species (Blanco *et al.* 2016, 2020; Young *et al.* 2012). These studies evaluated the occurrence and viability of tiny dispersed seeds, but the germination capability of seeds after parrot gut passage remains generally unknown. Seed dispersal effectiveness (SDE) is defined by the quantity (i.e. number of dispersed seeds) and quality (i.e. condition of the dispersed seeds) components (Schupp *et al.* 2010). To determine the SDE, quality component evaluation requires specific experiments testing the germination of gut-passed seeds compared with seeds directly collected from mature fruits. First, it requires an assessment of the potential effect of fertilization by faecal material that can enhance nutrient availability for seedling establishment (Traveset and Verdú 2002); though germination success may be reduced if faecal material facilitates the growth of detrimental fungi and bacteria (Meyer and Witmer 1998). Second, digestion helps to separate seeds from pulp, which can inhibit, reduce or enhance germination (Robertson *et al.* 2006). Therefore, the effects of fruit pulp and faecal material on final germination and viability should be tested to fully understand the factors governing endozoochorous seed dispersal interactions between parrots and their food plants.

In this study, we evaluated the role of the Austral parakeet (*Enicognathus ferrugineus*) as a seed disperser of the Southern barberry or calafate (*Berberis microphylla* G. Forst.; before *Berberis buxifolia*), a fleshy-fruited thorny bush of the family Berberidaceae inhabiting the understory of temperate forests in southern South America (Cabrera 1971). The two species share distribution across an extensive latitudinal gradient, although Austral parakeets show a large range at lower altitudes to the west of the Andes Mountains (Fig. 1) due to their long-distance seasonal movements (authors' unpublished data). In general, seeds of *Berberis* species show physiological dormancy (Baskin and Baskin 2014). Particularly, calafate is characterized by a deep physiological dormancy, since only some of the seeds become non-dormant and it only germinates after cold stratification that promotes seed germination in spring and summer 1 year after fructification and dispersal (Arena and Martínez-Pastur 1994). This adaptation can allow a delay in germination after the severe winter in the Andes Mountains, ensuring seedling survival (Cavieres and Arroyo 2000). A break in dormancy is not only promoted by temperature in this species, but also by chemicals such as sulfuric acid (Leod *et al.* 2015).

Here, we hypothesized that calafate seed germination is stimulated after gut passage of the Austral parakeet acting as a seed disperser. This hypothesis was tested experimentally using four seed germination treatments: (i) digested seeds, (ii) digested seeds with faecal extract, (iii) intact seeds from fruit and (iv) intact seeds from fruit with pulp. We predicted that the germination rate in seeds after gut passage would be greater compared with seeds from fruits. Calafate seeds from entire fruits and those excreted by frugivores remain subjected to extreme summer and winter environmental conditions, saprophytic organisms

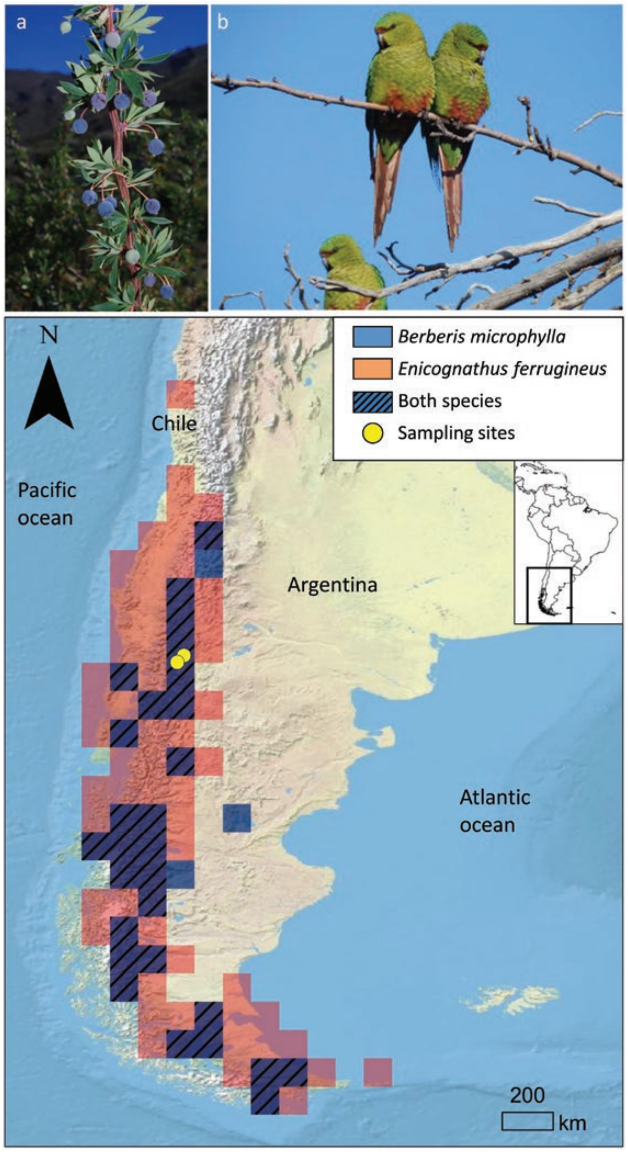

Figure 1: Location of the study area in north-western Argentinean Patagonia. Mature fruits of calafate (**a**) and faeces of Austral parakeets (**b**) were collected in two locations (Aucapan and Huechulafquen) of the Neuquén province (yellow circles). Distribution ranges of calafate (blue squares in the map) and Austral parakeets (red squares) are shown based on locations provided by GBIF (Global Biodiversity Information Facility 2019).

and consumers of pulp and seeds that degrade or destroy these matters over several months before germination. Specifically, seeds can spend several months under snow before germinating in the following spring after intense washing due to persistent rain and the spring thaw removing any faecal material or fruit pulp from the seeds. Therefore, we hypothesized that faecal material or fruit pulp should not affect seed germination after parakeet gut passage or seeds from entire fruits, respectively. This hypothesis predicts, therefore, that any difference in germination and viability between the two experimental groups can be mainly attributed to the potential enhancing effects of gut passage on breaking the physiological dormancy of seeds. Given that the Austral parakeet is currently the parrot species with the most southern distribution, and the only one that inhabits the Andean forests (Forshaw 1989), we discuss its role in the population dynamics of their food plants within the framework of ecological interactions and conservation of temperate forests of South America (Armesto *et al.* 2009).

MATERIALS AND METHODS

Study area

The study was conducted at two localities (Chiquilihuin-Aucapan and Huechulafquen) in the Neuquén province, north-western Argentine Patagonia (39°37′ S, 71°10′ W, 1000 m a.s.l.; Fig. 1). The area is a mixed temperate forest of monkey puzzle pine (*Araucaria araucana*) and lenga beech (*Nothofagus pumilio*), with a diverse understory of *Berberis* sp. and *Ribes magellanicum* along with several accompanying species (Oyarzabal *et al.* 2018). The weather is characterized by marked seasons with dry summers (December to February) with average temperatures of 17–19°C, and intense rain and snow periods in autumn and winter (April–September) with average temperatures of 7–8°C. Logging and pine plantations and livestock pasturing have increasingly destroyed and fragmented these forests, thus altering mutualistic seed dispersal interactions (Bustamante *et al.* 2003). This biome is of special conservation concern due to its highly endemic flora and fauna, and because of the occurrence of unique plant–animal interactions (Arroyo *et al.* 1996). The occurrence of mutualistic seed dispersal interactions in this biome is greater than in other temperate zones, being similar to that described in many tropical forests. At least 60% of the woody species of these forests produce fleshy fruits and rely on frugivorous birds and mammals to complete their reproductive cycle (Aizen and Ezcurra 1998). In spite of the importance of seed dispersal mutualisms in this biome, there is scarce and fragmentary information in terms of SDE.

Sampling procedure

During the spring (October) of 2016, we surveyed the study area looking for Austral parakeets. We particularly looked for foraging individuals in order to collect their faeces. Once parakeet flocks were located, we observed them, recorded their activity and attempted to collect fresh faeces below perching sites. A total of 29 fresh faeces were collected. Non-adjacent faeces were selected in order to avoid duplication of samples corresponding to the same individual. Every faecal sample was collected in a paper bag, dried rapidly to prevent fungal growth and stored at room temperature until arrival at the laboratory. On the same dates, a sample of mature calafate fruits were collected directly from the mother plants at every locality where we saw Austral parakeets feeding on this species. At the laboratory, calafate fruits were opened and seeds were separated and cleaned with deionized water to remove any remaining pulp, and dried with blotting paper. The number of seeds per fruit was counted, and the size of seeds was measured with digital callipers. Seeds from faeces were separated with the aid of binocular microscopes (×20), immediately washed with deionized water and gently dried with laboratory blotting paper. All seeds were stored in paper bags in a dark room at a constant temperature for 1 month until the beginning of the germination experiment (see details in Bravo *et al.* 2014).

To assess the fertilizing effect of faeces and the deinhibition effect of pulp fruit, extracts of parakeet droppings and calafate fruits were obtained following Bravo *et al.* (2014). A random sample of five mature fruits and five droppings were oven-dried (80°C; 72 h). Dry droppings (1.2 g) and dry fruits (1.5 g) were mashed, weighed and diluted with deionized water at 1:10.

Germination experiment

Calafate seeds are characterized by deep physiological dormancy (Baskin and Baskin 2014). The calafate produces berry fruits in late spring (October–December, Arena *et al.* 2012), with seeds germinating in following spring after breaking dormancy by low winter temperatures. Therefore, prior to incubation, seeds were subjected to a cold stratification at 4°C for 10 weeks to break physiological dormancy (Figueroa 2003).

Table 1: Percentage of faeces ($n = 29$) of Austral parakeets with presence of seeds, and number of faeces with seeds in parentheses, total number of seeds found in faeces, average seeds (± SD) per dropping and range of seeds (minimum and maximum)

	Berberis microphylla	Unidentified 1	Unidentified 2	Total
% of faeces with seeds (n)	65.5 (19)	20.7 (6)	3.5 (1)	72.4 (21)
Number of seeds	131	25	1	157
Seeds per faecal sample	6.9 ± 5.2	4.2 ± 5.0	1.0 ± 0	7.5 ± 7.4
Range	0–23	0–12	0–1	0–36

After cold stratification pre-treatment, seed samples were well mixed to obtain pools from different droppings (digested seeds) and from different mature fruits. In total, 100 digested seeds from faeces and 120 undigested seeds from fruits were set to germinate. Four treatments were carried out: DW (digested + water) treatment ($n = 50$ seeds), where digested seeds were germinated with deionized water (1.2 ml); DF (digested + faecal) treatment ($n = 50$ seeds), where digested seeds were germinated with parakeet faecal extract (1.2 ml); FW (fruit seeds + water) treatment ($n = 60$ seeds), where seeds from mature fruits were germinated with deionized water (1.2 ml); and FP (fruit seeds + pulp) treatment ($n = 60$ seeds), where seeds from mature fruits were germinated with pulp extract (1.2 ml).

Seeds were germinated in plastic Petri dishes (5.5 cm diameter) over two sheets of filter paper and were randomly distributed in a temperature-controlled chamber (Model G-21, Ibercex) programmed at 18/8°C with a photoperiod of 12/12 h. Dishes were incubated for 120 days. During the first 15 days, germination was recorded daily. From day 15 onward, germination was counted every 10–15 days. Radicle emergence was the criterion used to record a seed as germinated. Germinated seeds were removed. After each count, the Petri dishes were randomly moved to avoid a chamber position effect. After the first 90 days (when no further germination was observed), non-germinated seeds were set to germinate in 100 ppm solution of gibberellic acid (GA_3) for 30 days to promote germination of seeds which dormancy has not been broken by cold stratification. At the end of the incubation period, the viability of non-germinated seeds was checked by means of the tetrazolium test (in a solution of 1% solution of 2,3,5-triphenyl tetrazolium chloride for 24 h at 30°C) (Moore 1985).

Statistical analysis

Differences in seed size between digested seeds and seeds from fruits were analysed by a non-parametric Mann–Whitney U test, due to a lack of data normality. The germination process was characterized by final germination, seed viability percentage and germination time. Final germination was defined as the percentage of germinated seeds over 120 days corrected for viability (i.e. germinated seeds divided by total viable seeds). Seed viability percentage was defined as the number of viable seeds plus germinated seeds divided by total number of incubated seeds. Germination time was defined as the number of days until germination occurred. When a viable seed did not germinate at the end of the experiment, a value of 121 days (1 day greater than the observation time period) was assigned for the statistical analysis.

The treatment effect (DW, DF, FW and FP) on final germination and seed viability was tested by generalized linear mixed models (GLMM) with a binomial error distribution and logit link function, using the *lme4* package in R 2.15 (Bates *et al.* 2015). The treatment effect on germination time was analysed by GLMM with quasi-Poisson error distribution and log link function to deal with overdispersion. In both models, the experimental treatment was included as a fixed factor and Petri dish as a random one. A likelihood ratio test was used to assess the effect of each predictor. *Post hoc* comparisons between treatments were calculated with the Tukey test for all models using the *lsmeans* package in R 2.15 (Lenth 2016). Germination progress curves were plotted using the *drc* package (Ritz *et al.* 2015) to assess the process of germination of seeds included in each experimental treatment. All analyses were conducted with R, version 2.15.0. Results are reported as mean ± standard deviation (SD) in the text and mean ± SE in the figures.

Table 2: Statistical models for germination, viability and germination time of calafate seeds

	Estimate	SE	χ^2	df	P
Final germination					
Treatment			13.04	3	<0.05
Intercept	0.37	0.41			0.37
DF treatment	−0.83	0.62			0.18
FW treatment	−1.6	0.64			0.01
FP treatment	−1.44	0.65			0.03
Seed viability					
Treatment			2.97	3	0.40
Intercept	1.27	0.41			<0.05
DF treatment	−0.91	0.56			0.10
FW treatment	−0.15	0.59			0.81
FP treatment	−0.53	0.58			0.36
Germination time					
Treatment			18.8	3	<0.05
Intercept	96.58	3.58			<0.05
DF treatment	19.74	5.42			<0.05
FW treatment	19.91	5.34			<0.05
FP treatment	22.2	5.61			<0.05

Treatment was defined as digested seeds (DW treatment), digested seeds with faecal parakeet extract (DF treatment), seeds from mature fruits (FW treatment) and seeds from mature fruits with pulp fruit extract (FP treatment).

RESULTS

Most droppings contained seeds (72.4% of the total droppings, $n = 29$, Table 1). Seeds of three plant species were identified. Seeds of calafate were the most common, and were present in 19 droppings (65.5%), with an average (± SD) of 6.9 ± 5.2 seeds per dropping. Seeds of two undetermined species were recorded in six and in one droppings (20.7% and 3.4%, respectively), with an average of 4.2 ± 5.0 and 1 seed per dropping, respectively (Table 1). A large proportion of the seeds of the unidentified species were viable according to the tetrazolium tests (sp1 = 84%, n =25; sp2 = 100%, n =1).

Calafate fruits contained an average of 8.4 ± 2.6 seeds per fruit ($n = 20$). The fruit size was 7.59 (± 0.12) × 7.53 (± 0.11) mm ($n = 20$) and seed size was 4.32 (± 0.19) × 1.77 (± 0.09) mm ($n = 20$). The size of digested seeds was not significantly different from seeds from fruits (Mann–Whitney U test, length: $W = 227$, $P = 0.47$; width: $W = 197$, $P = 0.95$).

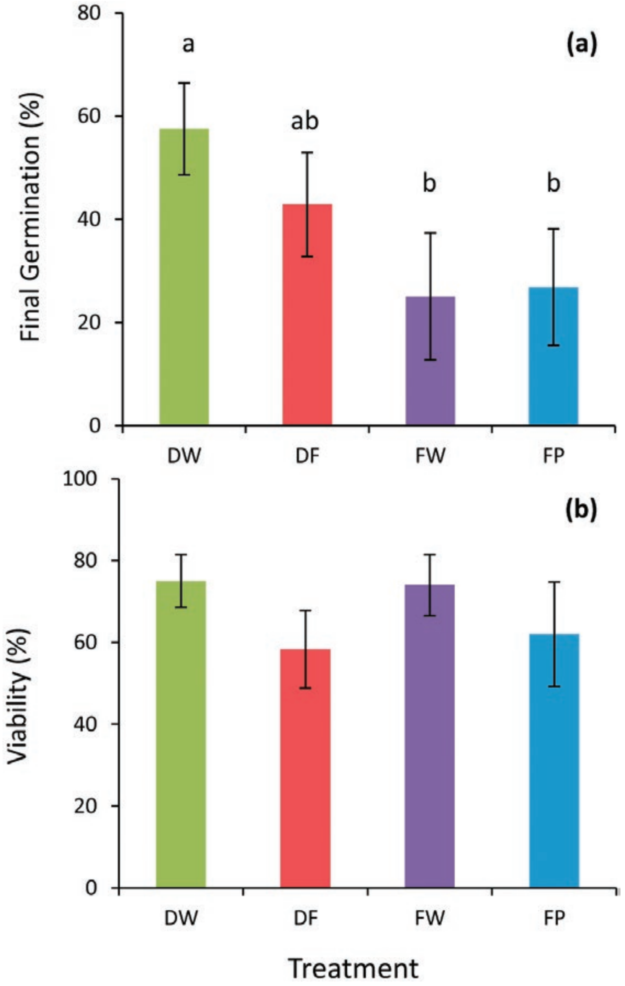

Figure 2: Differences in the proportion of (**a**) final seed germination and (**b**) seed viability, depending on experimental treatment of calafate seeds (see Materials and methods section for details and definitions). The four treatments are digested seeds (DW treatment), digested seeds with faecal parakeet extract (DF treatment), seeds from mature fruits (FW treatment) and seeds from mature fruit with pulp fruit extract (FP treatment). Significant between-treatment differences in *post hoc* tests are indicated by different lowercase letters. Mean ± SE are shown.

The analysis of final germination showed a significant effect of experimental treatment (Table 2 and Fig. 2a). Final germination was significantly higher in digested seeds (DW) than seeds from fruits (FW; Tukey test, Z = 3.06, P =0.01), and higher than those incubated with pulp extract (FP; Z = 2.71 P = 0.03). Faecal extract had no significant effect on final germination of digested seeds (DW vs DF: Z = 1.66, P = 0.35) (Fig. 2a). Pulp extract had no significant effect on final germination of seeds from fruits (FW vs FP: Z = −0.28, P = 0.99, Fig. 2a). Viability of seeds did not differ significantly between treatments (Table 2 and Fig. 2b).

Germination time differed significantly between treatments (Table 2). Digested seeds (DW, 96.3 ± 10.3, n = 45) started to germinate significantly earlier than seeds from the remaining treatments (DF: 116.3 ± 9.8, n = 35; FW: 117.0 ± 5.2, n = 37; FP: 118.7 ± 4.5, n = 31; *post hoc* test DW vs DF: T = −3.64, P < 0.05; DW vs FW: T = 3.73, P < 0.05; DW vs FP: T = 3.96, P < 0.05, Fig. 3). Digested seeds incubated with faecal extract (DF), seeds from fruits (FW) and pulp extract (FP) treatments did not increase germination until the gibberellic acid was added (Fig. 3).

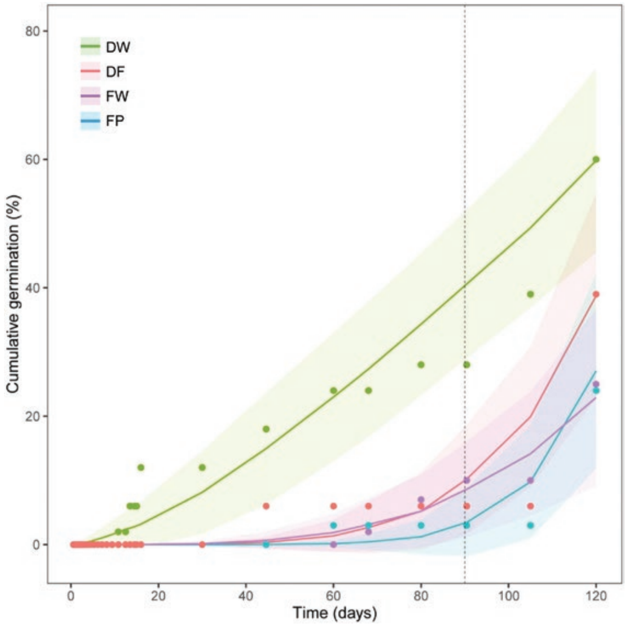

Figure 3: Adjusted curves (±95% confidence intervals) of cumulative germination in relation to time (days) for each experimental treatment group: DW: digested seeds; DF: digested seeds incubated with faecal parakeet extract; FW: seeds from mature fruits; FP: seeds from mature fruit incubated with pulp fruit extract. The dotted line shows the day that gibberellic acid (GA_3) was added to the Petri dishes. Points show mean observed cumulative germination for each treatment.

DISCUSSION

Our results indicate that the Austral parakeet acts as a legitimate endozoochorous disperser of calafate, a bush with physiologically dormant seeds. Passage through the parakeet gut can break the physiological dormancy of calafate seeds due to the potential effect of digestive acids on the seed embryo, thus enhancing seed germinability. The evaluation of fruit and seed trait effects on dispersal mode by each frugivorous species is essential to understand plant demography and population dynamics (Schupp *et al.* 2010; Snell *et al.* 2019; Traveset *et al.* 2014). Our results add to the growing evidence on the viability of seeds dispersed by parrots through endozoochory and stomatochory (Blanco *et al.* 2016, 2020; Tella *et al.* 2016b, 2019). Several studies have even highlighted an enhanced germination of seeds partially consumed and dispersed through stomatochory by different parrot species (Speziale *et al.* 2018; Tella *et al.* 2016a, 2019, 2020). However, to our knowledge, ours is the first study to experimentally evaluate seed germinability due to endozoochory by a wild parrot species, and one of the few that have documented endozoochory in the Andean forests of southern Patagonia (Mora and Soto-Gamboa 2011).

Most faeces of Austral parakeets contained calafate seeds (65.5%), at quantities of up to more than 20 seeds per dropping. Final germination of digested seeds dispersed by endozoochory (DW treatment) was higher and faster than intact seeds from fruit (FW treatment). Thus, the parakeet gut passage enhanced and stimulated germination without affecting seed viability. It is likely that the digestion process favoured the scarification of the seed coat facilitating the radicular protrusion. In this case, gut passage can also contribute to breaking the physiological dormancy of calafate seeds due to chemical changes produced in the seeds (internal or embryological) through the potential effects of the disperser gut acids. Germinability differences between digested and intact seeds from fruit support this hypothesis, as these differences remained even after the application of gibberellic acid. Due to the permeability of seeds with physiological dormancy, our results suggest that digestion could go beyond the physical changes to the seed coat in these species. Most studies on endozoochory generally do not consider the type of seed dormancy in each plant species (Soltani *et al.* 2018). Consequently, there is scarce information about how digestion influences seed physiological dormancy (Soltani *et al.* 2018). Further research is necessary to clarify the role of the digestion process in the breaking of seed physiological dormancy by frugivorous dispersers.

The faecal material surrounding defecated seeds diminished final seed germination compared with digested seeds, although the difference was not statistically significant. These results agree with the hypothesis that neither faecal matter nor fruit pulp provide seeds with any ecological advantages derived from enhancing germinability, but likely confer some disadvantage (Traveset *et al.* 2007). Germination time results supported this hypothesis as digested seeds (DW) started to germinate significantly earlier and faster than seeds of the remaining treatments, including digested seeds with faecal matter. Therefore, faecal matter and fruit pulp can negatively affect germination time in experimental conditions where these matters are forced to remain throughout the germination process. Previous studies have shown that seed germination can be negatively affected by the proliferation of fungi and bacteria, and by other factors associated with faecal material (Traveset *et al.* 2007). The lack of a negative effect of faecal extract on final germination, but on germination time, may be related to the seed dormancy mechanism of this species and its effect on delayed germination. In fact, the germination of calafate seeds occurs several months after fruiting and seed dispersal, when seeds are cleaned of any faecal or even pulp material, due to the washing effect of heavy rains and snowfall at high altitudes during the Austral autumn and winter in the Patagonian Andes. The slower germination time of digested seeds with faecal material suggests a detrimental effect of faecal matter. However, this effect does not reflect real conditions in nature, but only artificial laboratory conditions, as digested seeds do not remain with faecal material throughout the period from dispersal to germination. Overall, germination stimulation due to the break in physiological dormancy after gut passage can confer an important ecological advantage to calafate. This may be mainly because temperate forests of southern South America are characterized by the harsh rigorous climatic conditions, such as low temperatures and short periods suitable for seed germination and seedling recruitment (Figueroa 2003).

Among birds, only two passerines (*Elaenia albiceps* and *Turdus falcklandii*) have been recorded as potentially true seed dispersers in the temperate forests of southern South America (Aizen *et al.* 2002; Willson 1991), and there is only one previous study showing seed germination after ingestion by birds in these forests (Figueroa and Castro 2002). This previous study showed that a single plant species (*Luma apiculata*) out of five plant species tested showed a final germination significantly higher after digestion by birds. In our study, the more frequent and faster germination of digested calafate seeds compared with seeds from fruits suggests that Austral parakeets and calafate are involved in a close mutualistic relationship. Faster germination represents an ecological advantage due to early germination allowing rapid occupation in an ecosystem like the temperate forests of southern South America, which is characterized by a short period suitable for seed germination and seedling recruitment (Figueroa 2003). A limitation of our experiment is that it has been carried out only under lab conditions. Defecated seeds of some species can show different germination responses due to the experimental conditions (i.e. field vs common garden) (Rodríguez-Pérez *et al.* 2005). To more fully understand seed germination in plant species dispersed by parrots we encourage further investigation under wild conditions.

The Austral parakeet mostly feeds on seeds, flowers and fruits of the dominant tree and shrub species and many other plant and fungus species in Austral Andean forests (Diaz and Kitzberger 2006; Diaz *et al.* 2012). This species has been shown to be the main disperser and potential pollinator of the monkey puzzle pine (*Araucaria auracana*) (Gleiser *et al.* 2019; Speziale *et al.* 2018). However, its role in coevolutionary interactions in plant–animal networks has been generally neglected, as it is often only regarded as a plant antagonist (Gho-Illanes *et al.* 2015). Our study highlights a larger number of mutualistic interactions between Austral parakeets and their food plants than previously expected. These occur in a biome where interaction networks are considered highly specialized, and where plants and frugivorous communities are much less diverse than in tropical forests (Dalsgaard *et al.* 2017; Schleuning *et al.* 2012). In addition to the advantage of being dispersed by the parakeet, the earlier germination of digested seeds can favour seedling establishment after winter compared with seeds from intact fruits falling below the mother plant where establishment may be hindered. Furthermore, the Austral parakeet acts as a disperser vector for another two unidentified plant species whose viable seeds were found in faeces. In conclusion, our results highlight the relevant role of the Austral parakeet as a seed disperser of a dominant plant in the understory of Austral forests. Further research is needed that considers other plants consumed and potentially dispersed by Austral parakeets across their long-distance movements in southern South America.

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