Asiatic *Callosciurus* squirrels as seed dispersers of exotic plants in the Pampas

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Abstract

Seed dispersal by exotic mammals exemplifies mutualistic interactions that can modify the habitat by facilitating the establishment of certain species. We examined the potential for endozoochoric dispersal of exotic plants by *Callosciurus erythraeus* introduced in the Pampas Region of Argentina. We identified and characterized entire and damaged seeds found in squirrel faeces and evaluated the germination capacity and viability of entire seeds in laboratory assays. We collected 120 samples of squirrel faeces that contained 883 pellets in seasonal surveys conducted between July 2011 and June 2012 at 3 study sites within the main invasion focus of *C. erythraeus* in Argentina. We found 226 entire seeds in 21% of the samples belonging to 4 species of exotic trees and shrubs. Germination in laboratory assays was recorded for *Morus alba* and *Casuarina* sp.; however, germination percentage and rate was higher for seeds obtained from the fruits than for seeds obtained from the faeces. The largest size of entire seeds found in the faeces was 4.2 × 4.0 mm, whereas the damaged seeds had at least 1 dimension ≥ 4.7 mm. Our results indicated that *C. erythraeus* can disperse viable seeds of at least 2 species of exotic trees. *C. erythraeus* predated seeds of other naturalized species in the region. The morphometric description suggested a restriction on the maximum size for the passage of entire seeds through the digestive tract of squirrels, which provides useful information to predict its role as a potential disperser or predator of other species in other invaded communities.

Key words: Argentina, *Callosciurus erythraeus*, endozoochory, invasive species, mutualism.

Invasive species elicit changes in ecological, economic, and social systems as a result of their new interactions in the recipient environment (Simberloff et al. 2013; Blackburn et al. 2014). Polinization and seed dispersal are clear examples of these animal–plant interactions that promote species integration in the invaded community (Traveset and Richardson 2006). Fruit consumption and seed hoarding by vertebrate species may develop into mutualistic interactions if viable seeds are deposited in suitable conditions far from the parental plant (Howe and Smallwood 1982; Vander Wall et al. 2005). If the interacting species are both introduced, a beneficial outcome for one or both species would favor establishment, growth and/or spread, enhancing the invasion process (Simberloff and Von Holle 1999; Simberloff 2006). Although the importance of studying dispersal of exotic plants by introduced species has been highlighted, there is still unexplored, fertile ground between invasion and dispersal ecology, particularly for vertebrate-dispersed plants (Traveset and Richardson 2006; Westcott and Fletcher 2011).

Several rodent species are among the most damaging invasive species due to their impact on biodiversity and human activities and health (Jones et al. 2008; Harris 2009). Rodents have multiple effects on seed dispersal of exotic plants in the Pampas.
structure of the invaded community. Potential and effective dispersal by seed-caching squirrels has been reported (Paschoal and Galetti 1995; Wauters and Casale 1996; Vander Wall 2003; Moore and Swihart 2007; Xiao et al. 2009; Zong et al. 2010); however, few studies (López-Darias and Nagales 2008) have evaluated their role in endozoochory. Although 18 squirrel species have been introduced worldwide (Bertolino 2009; Jessen et al. 2010) the role of squirrels as seed dispersers and predators in invaded communities have remained largely uninvestigated.

Asiatic squirrels of the genus *Callosciurus* have been introduced in several European countries, South America and Asia (Lurz et al. 2013). Red-bellied squirrels *C. erythraeus* were imported into Argentina in 1970, where they have now established several populations due to their charismatic appeal and ability to cope with new habitats (Benitez et al. 2013; Guichón et al. 2015). Red-bellied squirrels have established in rural and urban habitats where arboreal vegetation is highly fragmented and mainly composed of exotic trees that provide food and nesting resources (Guichón and Doncaster 2008). These arboreal squirrels feed mainly on fruits, seeds, and leaves (Lurz et al., 2013); however, apart from general foraging habits, the new squirrel–plant interactions established in the invaded communities have not been evaluated and few reports exist on their native range (e.g. caching behavior: Chou et al. 2011).

In this study, we aim to evaluate squirrel–plant interactions taking place in the main invasion focus in Argentina, where exotic trees have facilitated squirrel success (Guichón and Doncaster 2008; Benitez et al. 2013). In particular, we studied the role of red-bellied squirrels as endozoochoric seed dispersers of introduced trees and shrubs in the Pampas Region. We analyzed the percentage and rate of germination of seeds obtained from squirrel faeces, their viability, and morphometric variables of entire and damaged seeds. Positive feedback between introduced species would exemplify facilitation due to animal–plant interactions that might affect the dynamics and structure of the invaded community.

### Material and Methods

#### Study area

We selected 3 study sites within the main invasion focus in Argentina, in the district of Luján (34°36’S, 59°11’W), North-eastern Buenos Aires province: 1) UNLu, the campus of the Universidad Nacional de Luján; 2) ACA, a recreational area 2.1 km from UNLu; and 3) Timón, another recreational area 10.3–12.2 km from the previous sites. These forested patches contain high squirrel density (15.3 ind/ha, CI 12.0–19.5, Benitez et al. 2013) and numerous arboreal species. Native grasslands of the Pampas region have been extensively modified by agriculture, livestock, and urbanization and are experiencing a woody invasion process (Ghersa et al. 2002). Squirrels use highly fragmented woodland patches in this rural and urban landscape (Guichón and Doncaster 2008). The arboreal vegetation is mainly composed of exotic species originally planted as windbreaks, for shade, ornamental purposes or timber and fruit production such as *Gleditsia triacanthos*, *Morus alba*, *Populus* spp., *Melia azedarach*, *Cuscuta* spp., *Capressus* spp., *Ligustrum* spp., *Pinus* spp., *Quercus* spp., and *Tilia* spp. Exotic trees are used by red-bellied squirrels for food and nesting. The climate is moist and temperate with a mean annual temperature of 16.6°C and annual precipitation averaging 951 mm (Goldberg et al. 1995).

#### Faeces and seed collection

Between June 2011 and July 2012, we collected squirrel faeces and leaves, flowers, fruits and seeds to make a reference collection of all tree and shrub species present within a 3-ha area at each study site. Squirrel faeces were collected seasonally during 15 days at 10 locations in each site. Each collection point consisted of 1 m² surfaces of either baited mesh traps at 1–2 m height hanging from tree branches or permanent tables that were carefully cleaned before each sampling season. The location of the faeces collection points was selected based on arboreal cover and daily movement of squirrels in each site. Squirrel faeces were identified in the field observing their size, color and shape, and by microscopic inspection in the laboratory of their rugosity and the presence of hairs of *C. erythraeus* (Pasola et al. 2005). All faeces collected in 1 trap or table per season were pooled into 1 sample that was submerged in distilled water for 30 min to facilitate pellet disintegration. Each sample was then filtered to retain fragments >500 μm, which were carefully observed under a binocular magnifying glass (10×) in order to separate all entire and damaged seeds that were identified using the reference collection. We measured the following morphometric variables of all seed species found in the faeces using 50 seeds per species from the reference collection: weight (W), length (L), width (Wi), and length to width ratio (L/Wi). Entire seeds were refrigerated until we initiated the germination assay (<1 month in all cases).

#### Germination and viability assay

We evaluated seed germination capacity by sowing individually all entire seeds found in squirrel faeces in petri dishes (26 × 11 mm) with humid filter paper for a maximum of 30 days. We also sowed seeds belonging to the same species but obtained directly from the fruits within the study sites as a control treatment. During the assay, seeds were kept in a germination room (20–30°C, 12–12 h light–darkness) and observed every day to check for root emergence indicating seed germination. We calculated the germination percentage as the number of germinated seeds over the total number of sown seeds × 100 and the germination rate as the time elapsed until the germination of 50% of the sown seeds (Bewley and Black 1994). We analyzed differences in the proportion of germinated seeds obtained from the faeces and the control treatment using a test of homogeneity with the Yates corrected chi-square statistic.

We evaluated the viability of the seeds that did not germinate during the 30 days assays using a standard bioindicator (2,3,5 triphenyl tetrazolium chloride (TTC)) that detects seed viability by staining the embryo tissue pink/red (Moore 1985). We determined seed viability observing seed coloration using microscope amplification in comparison with positive control seeds collected in the study sites.

#### Results

##### Faeces and seed collection

We collected ≥ 40 squirrel faeces in 10 samples per site in each season that yielded a total of 883 faeces in 120 samples throughout the study period. We found damaged seeds in 26% of the samples that corresponded to *Ligustrum sinense*, *L. lucidum*, and *Melia azederach*, whereas 21% of the samples contained 226 entire seeds of 4 exotic species plus 2 seeds that could not be identified (Table 1). Most (92%) entire seeds were found in spring and corresponded to only one species, *Morus alba* (Table 1). Therefore, only a small
fraction of the 49 tree and shrub species recorded in the study sites, which included just 3 native species, were present in the faeces.

Entire seeds found in squirrel faeces had a mean maximum size of 4.2 × 4.0 mm, whereas damaged seeds had at least 1 larger dimension (> 4.7 mm) (Table 2). High overlap between entire and damaged seeds was found in weight and length/width ratio (Table 2).

Germination and viability assay

Only 2 of the 4 species sown germinated during the assay, *Casuarina* sp. and *Morus alba* (Table 1). The 2 unidentified seeds did not germinate. Taking the 2 species together, seeds obtained from faeces (digested) showed a lower germination percentage (64%) than the seeds obtained from the fruits (control) (94%) (χ² = 68.77, P < 0.001, df = 1); the same pattern was recorded in both species (Table 1). Germination rate was higher for the control treatment (3 days) than for the digested seeds (5 days) (Figure 1). All digested and control seeds that did not germinate during the assay were not viable (Table 1). Approximately, 50% of the 81 digested seeds that did not germinate were nonproductive seeds, that is empty seeds, poorly developed embryo, infected by fungi, whereas only 29% of the control seeds showed any of these conditions.

Table 1. Number of faeces found in the 30 samples collected every season throughout the sampling year at 3 study sites within the main invasion focus in the province of Buenos Aires, Argentina

| Season | Number of faeces | Species          | Germinated (%) | Viable (%) |
|--------|------------------|------------------|----------------|------------|
| Winter | 269              | *Casuarina sp.*  | 4/8 (50%)      | 0/4        |
| Spring | 186              | *Morus alba*     | 141/209 (67%)  | 0/68       |
| Summer | 172              | *Schinus molle*  | 0/7 (—)        | 0/7        |
| Autumn | 236              | *Pyracantha sp.* | 0/1 (—)        | 0/1        |

Number of germinated seeds over the total number of seeds sown for seeds obtained from the faeces (digested) and from the fruits (control); total seeds sown = 452. Number of viable seeds over total number of seeds tested for digested and control seeds that did not germinate in the assay; total seeds tested = 95.

Table 2. Growth form, fruit type, and morphometric measures (mean ± SD) of the 50 seeds measured for each species of entire and damaged seeds found in the faeces

| Species          | Growth form | Fruit type        | Width (mm) | Length (mm) | Length/Width | Weight (mg) |
|------------------|-------------|-------------------|------------|-------------|--------------|-------------|
| *Entire seeds*   |             |                   |            |             |              |             |
| *Casuarina sp.*  | Tree        | Woody infrutescencia | 1.6 ± 0.17 | 3.8 ± 0.2  | 2.4 ± 0.2    | 0.68 ± 0.15 |
| *Morus alba*     | Tree        | Multiple fruit     | 1.7 ± 0.14 | 2.4 ± 0.12 | 1.4 ± 0.14   | 1.9 ± 0.5   |
| *Schinus molle*  | Tree        | Drupe              | 4.2 ± 0.23 | 4.0 ± 0.22 | 1.0 ± 0.07   | 27.06 ± 5.29|
| *Pyracantha sp.* | Shrub       | Pome               | 4.1 ± 0.10 | 2.3 ± 0.14 | 1.4 ± 0.09   | 2.5 ± 0.48  |
| *Damaged seeds*  |             |                   |            |             |              |             |
| *Ligustrum sinens* | Shrub     | Drupe             | 2.7 ± 0.30 | 4.7 ± 0.45 | 1.8 ± 0.13   | 9.16 ± 1.5  |
| *Ligustrum lucidum* | Tree    | Drupe             | 2.9 ± 0.28 | 5.9 ± 0.64 | 2.0 ± 0.18   | 11.6 ± 2.1  |
| *Melia azedarach* | Tree       | Drupe             | 3.0 ± 0.35 | 7.3 ± 0.54 | 2.4 ± 0.36   | 25.2 ± 5.03 |

The role of *C. erythraeus* as a seed-disperser has been reported through seed caching and epizoochory (Xiao et al. 2009; Cao et al. 2011; Zhou et al. 2013); however, this is the first study to analyze its role as endozoochorous disperser. Two of the four species of entire digested seeds did germinate, *Morus alba* and *Casuarina* spp. *Morus alba* is from China though it is extensively cultivated and naturalized in north and central Argentina (Ghersa et al. 2002). Its widespread distribution in Argentina mainly results from seed dispersal by birds (Delacchi 1989). This constitutes the first report of mammal mediated dispersal of *M. alba* in the region; however, dispersal of this species by other mammals such as *Sus scrofa* (Dovrat et al. 2012) and frugivorous bats (Galindo-González 1998; Jorda et al. 2011) was reported in other regions. As far as we know, this interaction between *C. erythraeus* and *M. alba* has not been reported previously in either native or introduced squirrel ranges worldwide. Seeds of *Casuarina* spp., species native to Australia and cultivated in Argentina, are mainly dispersed by wind and water though in their native range, dispersal by the cockatoo *Calyptrorhynchus lathami* has also been reported (Wheeler et al. 2011). *Callosciurus erythraeus* is the first disperser reported for *Casuarina* spp. in our study region.

The passage through the digestive tract of *C. erythraeus* diminished both germination percentage and rate in comparison with the seeds obtained directly from the fruits. However, the relatively large number of seeds that germinated after ingestion by squirrels could provide an advantage if seeds are deposited in suitable sites, far from the parental tree (Schupp et al. 2010; Westcott and Fletcher 2011). Comparison with other dispersal agents to evaluate facilitation by these incipient new interactions should consider plant benefit resulting from deposition patterns of seeds, field germination success, and disperser abundance and habits regarding feeding and movement (Kitamura et al. 2006; Schupp et al. 2010). Furthermore, the identity and relative importance of each squirrel–plant interaction will largely depend on plant availability in the invaded community (Traveset 1998; Busch et al. 2012), as has been reported for *Oryctolagus cuniculus* that could disperse 8–50% of the available species depending on the community type (Della Fiore et al. 2009). This reinforces the need for further studies in other areas invaded by *C. erythraeus* in Argentina (Benitez et al. 2006).
2013; Guichón et al. 2015) and in other countries (Lurz et al. 2013) to detect new interactions and their impact on plant recruitment and community structure.

The potential to disperse and depredate 7 tree and shrub species represents only 14% of the available species in the study sites, although seed predation could be underestimated given that we did not conduct microhistological analysis of the faeces. In previous studies conducted in Argentina, 29% of the species available in the study sites were consumed by squirrels, and we also found seeds of M. azedarach, Pyracantha sp., and L. lucidum though seeds of another 16 species were recorded, including Juglans australis, Acer negundo, Fraxinus excelsior, Cupressus spp., and Crataegus monogyna (Zarco, Benitez and Guichón, unpublished data). Callosciurus erythraeus would be dispersing a small number of species according to our results, and thus it could play a more important role as seed predator. Seed predation by C. erythraeus has been reported in its native range (Chou et al. 2011; Sethi and Howe 2012) and other introduced areas where it consumes seeds avoided by native species (Tamura and Miyashita 1984; Corlett 2005). Other Callosciurus species, C. notatus and C. prevostii, also play a more important role as seed predators than dispersers in their native range (Becker and Wong 1985). Seed size could explain whether squirrels may act as a disperser or predator given that it affects ingestion mode, quantity of food consumed, dispersal form and the role of the disperser (Gautier-Hion et al. 1985; Montaldo 2005). Our results suggest that the maximum dimension for squirrels to digest entire seeds ranges 4.2–4.6 mm, though new studies evaluating a larger number of seed species are necessary to predict potential impact.

Our study has identified new interactions between introduced species; further studies may detect others in areas where a different set of species is available in the recipient community. We reinforce the importance of studies that may detect the role of squirrels as dispersers of exotic species or predators of native ones given the proximity of C. erythraeus to areas of high conservation value, such as the Natural Reserve Otamendi and the Delta Region of the Paraná river (Benitez et al. 2013; Guichón et al. 2015). Knowledge regarding squirrel–plant interactions in the invaded communities and size of seeds either dispersed or predated would contribute to the evaluation of potential impacts of squirrel establishment on species of conservation value or on species planted for agricultural production or ornamental purposes.

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Figure 1. Proportion of seeds germinated throughout the 30-day assay for seeds obtained from the faeces (digested, lower line) and from the fruits (control, upper line).
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