Gut passage time and viability of seeds consumed by Australian marsupials

Gabrielle Beca*, Bryony Palmer, Leonie E. Valentine, Todd E. Erickson, Richard J. Hobbs

A School of Biological Sciences, University of Western Australia, Crawley, 6009, WA, Australia
B Kings Park Science, Department of Biodiversity, Conservation and Attractions, Kings Park, 6005, WA, Australia

* Corresponding author. Email: gabrielle.beca@research.uwa.edu.au

Publication information:

https://doi.org/10.1071/AM20063
Abstract
Many Australian mammals consume seeds, but their role in seed dispersal has not been well explored. Here, we investigated the mean retention time and the post-consumption germination capacity of Australian seeds (Acacia acuminata, Dodonaea viscosa and Gastrolobium calycinum) likely to be consumed by quenda (Isoodon fusciventer) and woylies (Bettongia penicillata ogilbyi). Mean excretion times were 14 hours for quenda and 24 hours for woylies, but some seeds were retained in their digestive passages for up to 39.5 and 55.5 hours, respectively. Viable seeds of all plant species were retrieved from both species’ scats and only G. calycinum seeds ingested by quenda (62%) had a significantly higher germination percentage than control seeds (34%). Our results show that viable seeds are deposited in the scats of quenda and woylies, indicating that these species may play a role in seed dispersal.

Keywords: endozoochory, granivory, seed dispersal, seed germination, potoroid, peramelid.

Introduction
Endozoochory, the dispersal of plant seeds via ingestion by a vertebrate animal, can be advantageous for plants in several ways. Seeds may be dispersed at a greater distance from the parent plant than other forms of seed dispersal (Carlo et al. 2007), reducing both competition for resources and predation rates (Janzen 1970). Deposition in faeces may provide moisture and nutrients, enhancing germination and growth rates (Traveset et al. 2007). Additionally, seeds consumed by animals sometimes have increased germination rates (Cantor et al. 2010) due to the alleviation of seed dormancy (Traveset 1998).

Seeds form an important dietary component for many Australian mammals (Quin 1985; Murray et al. 1999; Gibson 2001; Bice and Moseby 2008), and some groups, such as bettongs, disperse seeds via scatter-hoarding (Murphy 2009; Chapman 2015). Australian seed-eating mammals are recognised as potentially important seed predators (Ballardie and Whelan 1986; Auld and Denham 1999; Mills et al. 2018), but the extent to which they disperse seeds via endozoochory has received little research attention (Williams et al. 2000; Cochrane et al. 2005).

Physical seed dormancy is an adaptive trait that acts to prevent germination when environmental conditions are suitable for germination, but not favourable for seedling survival (Merritt et al. 2007). Australian seeds in the genera Acacia, Dodonaea and Gastrolobium, for example, exhibit this adaptation and germinate only after exposure to high temperatures (Sweedman and Merritt 2006). Previous research suggests that germination capacity is
increased if gut passage acts to break dormancy inhibitors such as removal of surrounding fruit pulp (Petre et al. 2015), or through seed coat scarification (Traveset 1998). However, the effect of consumption by marsupials on dormancy in Australian seeds is unknown.

The time it takes an animal to pass a seed through its digestive tract (i.e. its gut passage time) will affect its effectiveness as a seed dispersal agent. Longer gut passage times may result in the dispersal of propagules over greater distances (Petre et al. 2015) and scarification in the gut can increase germination capacity by breaking seed dormancy. However, if gut passage times are too long seeds may be damaged or lose viability (Traveset et al. 2016). Whilst the gut passage times for several Australian mammals have been studied using markers that bind to solutes or small particles (e.g. Wallis 1994; Moyle et al. 1995; Gibson and Hume 2000), there has been no research into the passage time of seeds.

In this paper we use two species known to consume seeds, quenda (Isoodon fuscivent) and woylies (Bettongia penicillata ogilbyi), to investigate endozoochory in Australian mammals. We sought to determine the rate at which these species pass seeds through their digestive tract and the germination capacity of seeds retrieved from their scats. We tested the following questions: (1) How long do quenda and woylies take to pass seeds through their digestive systems? and (2) Is the germination capacity of seeds retrieved from scats of quenda and woylies higher than unconsumed seeds?

Materials and methods

Two female adult quenda (I. fuscivent) and a male and female adult woylie (B. penicillata) held in captivity at Native Animal Rescue (NAR), Malaga, Western Australia were used in this experiment. During the experiment, conducted in June 2019, the animals were housed in individual enclosures approximately 2 x 3 m in size. The floors of the enclosures were cleared of leaf litter and excess foliage to facilitate the location of scats. A hollow log or nest box, and a small bundle of fresh foliage were retained in each enclosure.

The animals’ regular feed consists of fruit, vegetables and a boiled egg with the addition of mealworms, insectivore mix and aviary seed (quenda) or herbivore pellets (woylies). For one week prior to, and during the experiment, seeds and fruit containing seeds were substituted with other items (e.g. similar fruit or vegetables without seeds). Feed was provided daily in the late afternoon and remaining food was removed each morning. Water was provided ad libitum throughout.

We used commercially sourced seeds (Nindethana Australian Native Seeds – King River, WA) of three native species previously recorded in the diet of Potoroids or Peramelids: Acacia
acuminata, Dodonaea viscosa and Gastrolobium calycinum (Christensen 1980; Bice and Moseby 2008) (Table S1). Prior to the start of the experiment, we determined the viability of the seeds using a Faxitron MX-20 Digital X-Ray Cabinet (Tucson, Arizona, USA). Seeds that appeared uniformly white/grey in the digital imagery were deemed to be viable (Erickson and Merritt 2016). Seeds that appeared shrivelled/empty or abnormal were deemed non-viable and were discarded. The viable seeds were not treated to break physical dormancy so that we could investigate how this was affected by consumption by our study species. The germination capacity of control seeds (i.e. seeds not consumed by the study animals) was determined in the laboratory by incubating 100 seeds of each species at 15°C with a 12 hour light-dark cycle, using water agar as a germination medium (Merritt 2006). Twenty-five seeds of a single species were sown onto each petri dish. Prior to sowing, seeds were rinsed in a 30 gL⁻¹ bleach solution and sterilised water to reduce fungal contaminants (Merritt 2006). The number of germinating seeds was then counted weekly for four weeks.

At the commencement of the feeding experiment, 150 viable seeds (50 of each species), taken from the same seed pool as the controls, were mixed into each animal’s usual feed. The modified feed was provided to the animals at 1600 h on the first day of the experiment and the remains removed at 0700 h the following morning. Remaining feed and the enclosures were searched for unconsumed seeds or seed caches so that the exact number of seeds consumed by each animal (i.e. the number of seeds found subtracted from 150) could be determined (Table 1).

Scat collection commenced at 2400 h (approximately 8 hours after presentation of the seeds) and was conducted at approximately 0700, 1600 and 2400 h for four consecutive days. A final scat collection event was conducted at 0700 h on day five, giving a total of 14 collection events (over 107 h) for each animal. This time period was based on marker excretion times of three species of potorine marsupials (Wallis 1994). During each scat collection event, the floor of the enclosures, the refuges and fresh foliage were searched carefully for scats or loose seeds by one or two observers, and the start time and duration of search were recorded. The midpoint between the end and start times of consecutive checks was used as the excretion time for calculations, as per Wallis (1994). Collected scats were placed into paper bags and stored in a well-ventilated location at room temperature until the conclusion of the experiment. The samples were dried at 35°C for six days and then stored at room temperature until the scat analysis was conducted.

All scats were examined for seeds. Scats were soaked in water and then carefully teased apart. Whole seeds, with or without the elaiosomes, were removed, sorted by species and dried.
Where the number of retrieved seeds was less than the number consumed by the animals, we assumed the missing seeds were destroyed during the digestive process. The viability of the retrieved seeds was retested using the x-ray method described above. We performed a germination trial, including the seed sterilisation step, as described above, on all retrieved seeds that appeared viable using separate petri dishes for each animal, seed species and collection event combination. Differences in the germination capacity of viable seeds retrieved from scats and control seeds were assessed using Chi-square analyses in R statistical software (R Core Team 2019). We used the percentage of seeds that germinated as the response variable.

**Results**

The animals consumed most of the seeds presented to them (mean 71%, range 35-97%; Table 1) but many of the consumed seeds were damaged or destroyed. Seed fragments were found frequently in the scats but could not be identified to species and were discarded. Overall, less than half of the consumed seeds were retrieved whole, with or without the elaiosome, but recovery rates were highly variable between individuals (mean 46.8%, range 9-83%; Table 1).

The peak excretion (54% of seeds) for quenda occurred between 4-11.5 hours (Fig. 1). For woylies, the peak excretion (59% of seeds) occurred between 19.5-24 hours (Fig. 1). Mean gut passage time was similar to the time of peak excretion for both species: 14±3 hours for quenda and 24±2 hours for woylies.
Fig. 1. Relationship between the retention time (in hours) and the cumulative percentage of viable seeds of *A. acuminata*, *D. viscosa*, *G. calycinum* recovered from scats of (a) quenda and (b) woylies. The experiment was continued until 103.5 h after consumption, but the last seeds were recovered from the scats at 39.5 h (quenda) and 55.5 h (woylies).

Of the seeds retrieved, 100% percent of *A. acuminata*, 96% of *D. viscosa* and 97% of *G. calycinum* seeds (97% overall) were viable after consumption by quenda. For seeds consumed by woylies, 100% of *D. viscosa*, 87% of *G. calycinum* and 80% of *A. acuminata* seeds (88% overall) remained viable. Germination of *A. acuminata*, *D. viscosa* and *G. calycinum* occurred in both control and ingested seeds (Table 2). There was no significant difference between the germination capacity of *A. acuminata* seeds ingested by quendas or woylies and the controls (Table 2). *Dodonaea viscosa* seeds ingested by quenda had a significantly lower germination capacity compared to the controls, but there was no difference between the seeds ingested by woylies and the control seeds (Table 2). *Gastrolobium calycinum* seeds ingested by quenda had a significantly higher germination capacity than the control seeds, but seeds consumed by woylies had a significantly lower germination capacity than the control (Table 2).

Table 1. Number of seeds of each species used in the experiment, their consumption (i.e. number of seeds found subtracted from presented seeds), recovery, viability and germination capacity. Numbers in brackets represent the percentage of the previous section’s total.

<table>
<thead>
<tr>
<th>Seeds presented</th>
<th>Seeds consumed</th>
<th>Seeds recovered</th>
<th>Seeds viable</th>
<th>Seeds germinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>D</td>
<td>G</td>
<td>Total</td>
</tr>
<tr>
<td>Quenda 1 (%)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Quenda 2 (%)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Woylie 1 (%)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Woylie 2 (%)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>150</td>
</tr>
</tbody>
</table>

A – *Acacia acuminata*, D – *Dodonaea viscosa*, G – *Gastrolobium calycinum*

Table 2. Number and percentage of viable seeds recovered from scats that germinated. Significant differences between viable seeds recovered from scats of quenda or woylies and the controls are indicated by bold font.

<table>
<thead>
<tr>
<th>Seed species</th>
<th>Control germinants</th>
<th>Germinants</th>
<th>Quenda</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>Germinants</th>
<th>Woylie</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia acuminata</em></td>
<td>15 (15%)</td>
<td>2 (7.7%)</td>
<td>0.12</td>
<td>0.72</td>
<td>2 (8.3%)</td>
<td>1.91</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dodonaea viscosa</em></td>
<td>51 (51%)</td>
<td>5 (12.8%)</td>
<td>22.84</td>
<td>0.001</td>
<td>10 (45.4%)</td>
<td>0.32</td>
<td>0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gastrolobium calycinum</em></td>
<td>34 (34%)</td>
<td>18 (62.1%)</td>
<td>8.19</td>
<td>0.004</td>
<td>5 (18.5%)</td>
<td>4.57</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Our study shows that viable seeds, capable of germinating under laboratory conditions, are deposited in the scats of quenda and woylies. Only forty-two percent of the consumed seeds were retrieved whole from the scats suggesting that quenda and woylies may act as seed predators. However, the high viability and germination of some seeds retrieved from the scats indicate that these species may also play a role in seed dispersal through endozoochory.

Research on endozoochory in Australian marsupials is rare, but consumption by *Potorous gilbertii* and *Setonix brachyurus* increased germination in *Billardiera fusiformis* (Cochrane *et al.* 2005). Similarly, we found that consumption by quenda increased germination in *G. calycinum*. However, overall, the germination capacity of seeds consumed by woylies and quenda was half that of the control seeds. Physically dormant seeds, such as those used in our study, require high temperature cues or mechanical scarification to render the seed coat permeable to water and allow germination to occur if conditions are suitable (Sweedman and Merritt 2006). Mastication or exposure to stomach acids may break physical seed dormancy, but can also damage seeds (Traveset *et al.* 2007). We showed that dormancy was alleviated in the seeds that experienced the shortest gut passage time, *G. calycinum* consumed by quenda. However, the low number of intact seeds we recovered, and similar or lower germination capacity in the remaining species, indicates that consumption by quenda and woylies most often damages seeds.

Although consumption by woylies or quenda improved germination capacity in only one seed species, endozoochorous dispersal offers other advantages to seeds. The deposition of seeds within scats may provide nutrients and protection from seed predators (Traveset *et al.* 2007) and is more likely to occur to favourable locations (Carlo *et al.* 2007). For example, quenda and woylies forage extensively for subterranean food resources (Garkaklis *et al.* 2004; Valentine *et al.* 2012) and often defecate next to their foraging pits (B. Palmer pers. obs.). These scats may be covered by the ejected soil during pit creation or fall into the pits. Seeds in foraging pits are more likely to germinate (Valentine *et al.* 2017), exhibit higher growth rates (Valentine *et al.* 2018) and are less likely to be predated (Radnan and Eldridge 2017).

The mean excretion times we recorded for quenda and woylies are within the range of published values for marker excretion times for other Peramelids and Potoroids (Wallis 1994; McClelland *et al.* 1999), suggesting that standard digestive rate methods provide reasonable estimates of seed retention times. Most seeds in this study were passed quickly, with excretion rates peaking at 11.5 and 24 hours for quenda and woylies, respectively. Both species, however, retained some seeds for at least 39.5 hours. The gut passage times we recorded, combined with
their home range sizes (quenda 5 ha, Van Dyck and Strahan 2008; woylies 65 ha, Yeatman and Wayne 2015), indicate that quenda and woylies may disperse seed over substantial distances. This may confer advantages, such as reduced competition with parent or sibling plants, to those seeds that remain viable after excretion and serve to counteract the negative impact of seed predation on plant populations.

Reintroducing mammals could assist restoration efforts by increasing the dispersal of native plants (Cantor et al. 2010; Genes et al. 2019). However, animals can also disperse exotic species (Dovrat et al. 2012), and this should be considered when predicting the ecological outcomes of a translocation. Given the gut passage times we recorded, during wild-to-wild translocations quenda and woylies would probably excrete most seeds consumed prior to their capture during the holding or transport stage, but some seeds could be excreted at the release site. We suggest that scats excreted during holding and transport are retained and disposed of where germination cannot occur, unless dispersal of plant species from the source site is desirable. Where the source site supports exotic species absent at the release site, post-release monitoring for seedling recruitment of species of concern may be warranted.

Our study used only four captive animals and may not be representative of what happens in natural systems. For example, bettongs and bandicoots feed on mycorrhizal fungi and the co-occurrence of seeds and mycorrhizal fungal spores in scats may alter germination or growth rates (Tay et al. 2018). Field-based studies are required to determine how often free-living quenda and woylies consume seeds, and if this seed can germinate after consumption. Investigations on how consumption by other Australian mammals affects seed viability and germination capacity of a wide range of seeds, especially those already known to be part of their diet (Morton 1985), would also be useful. Our research demonstrates that endozoochory by Australian mammals is possible; future research will hopefully determine whether it contributes significantly to vegetation dynamics in natural settings.

Acknowledgements

This project was undertaken in accordance with the regulations of University of Western Australia Animal Ethics Committee (RA/3/100/1655). We thank Native Animal Rescue staff and volunteers, and in particular L. Carson, for allowing us access to their facilities and animals and assisting with the study. We also thank the Kings Park Science team at the Department of Biodiversity, Conservation and Attractions for the use of the seed laboratory, particularly D. Merritt for his assistance with the seed viability and germination testing. G. Beca and B. Palmer were supported by a University of Western Australia Postgraduate Award and by the Holsworth...
Wildlife Research Endowment & The Ecological Society of Australia. G. Beca, B. Palmer and L. Valentine received support from the Australian Government’s National Environmental Science Program through the Threatened Species Recovery Hub.

Conflicts of interest
The authors declare no conflicts of interest.

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