SHORT COMMUNICATION

Germination after simulated rat damage in seeds of two endemic Hawaiian palm species

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Seed predation by native and alien rodents can limit plant recruitment and ultimately affect forest dynamics and composition (Campbell & Atkinson 2002, Côté *et al.* 2003, Hulme 1998, Sánchez-Cordero & Martínez-Gallardo 1998). Even partial consumption of seeds by predators may affect plant community structure, though its importance is poorly understood (Steele *et al.* 1993, Vallejo-Marín *et al.* 2006). Despite consumption of relatively large portions of seeds by herbivores, seeds can retain their ability to germinate if the embryo remains intact (Dalling & Harms 1999, Janzen 1972, Mack 1998). Germination of damaged seeds may be accelerated or prolonged (Karban & Lowenberg 1992, Koptur 1998, Vallejo-Marín *et al.* 2006). Damage by seed pests also facilitates ageing stress; which manifests as decreased seedling vigour, decreased seed viability, lower germination percentages and slower germination rates (Priestley 1986).

Palaeontological evidence indicates that Polynesia lacked rodents until they were introduced by human colonists. Beginning about 3000 y ago, the Polynesian rat (*Rattus exulans* Peale) was transported and became established with Polynesian settlers (Matisoo-Smith & Robins 2004, Wilmshurst *et al.* 2008). Black rats (*Rattus rattus* L.) invaded the Pacific after European contact. Introduced rats depredate seeds of various plant species throughout the South Pacific (Allen *et al.* 1994, McConkey *et al.* 2003) and may have played a significant role in the prehistoric collapse of native palm forests in Hawaii (Athens *et al.* 2002) and on Easter Island (Rapa Nui, Hunt 2007). *Rattus rattus* has been implicated as

the primary contemporary consumer of dispersed fruits and seeds of *Pritchardia hillebrandii* (Kuntze) Becc. (Shiels & Drake 2007), a species of conservation concern, and *Pritchardia kaalae* Rock (Arcand *et al.* 2003), a federally listed endangered species (USFWS 2003). Middens of partially eaten fruits and seeds have been found within a relic *P. kaalae* population (Pérez, pers. obs.). However, it is not known if partially eaten seeds of *Pritchardia* retain the ability to germinate and become established.

We tested the extent to which rats damage seeds of *P. hillebrandii* and *P. kaalae*, and whether germination of these two palm species is affected by simulated seed herbivory (partial removal of endosperm). Specifically, we tested the hypotheses that (1) final germination percentage (FGP) decreases and (2) mean time to complete germination (MTG) increases with increasing levels of endosperm removal.

Pritchardia fruits were collected between July and September, 2002. Ripe fruits of *P. hillebrandii* were harvested randomly from nine cultivated trees growing in Kalāheo, Kaua'i (21°54′N, 159°35′W, elev. 29 m). Fruits of *P. kaalae* were collected from 57 trees of a wild population growing within lowland mesic forest on the 'Ohikilolo Ridge, O'ahu (21°30′N, 158°11′W, elev. 933 m). Fruits were stored in open trays on a laboratory bench-top (Honolulu, Hawai'i) at about 25 °C. Experiments began approximately 3 wk after collection. Seeds were extracted from pericarps by squeezing drupes with pliers and then soaked in a 70% ethanol solution for 5 min followed by a 20-min soak in 0.5% sodium hypochlorite to reduce chances of microbial infection. Seeds (n = 15) of *P. hillebrandii* are smaller, averaging 1.1 g (dry mass) and 0.8 cm^3, than *P. kaalae* (averaging 4.0 g, 6.2 cm^3).

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Four levels of endosperm removal were applied randomly to seeds. The treatments were no damage (0% mass removed), minimally damaged (*c.* 15%), moderately damaged (*c.* 45%) and severely damaged (*c.* 75%). To keep the embryo intact, endosperm removal began on the hemisphere opposite the operculum. Endosperm removal was achieved by cutting seeds with hand-held ratchet pruners (Corona Clipper, Inc. Corona, CA) and a Dremel Rotary Tool (Model 750, Dremel, Racine, WI).

Pritchardia seeds possess only morpho-physiological dormancy and germination may be spread over 21 wk after shedding. Therefore, dormancy in the test species was alleviated by removing seeds from fruits and using temperature, moisture, and light conditions outlined previously (Pérez *et al.* 2008). For both species, 15 seeds were treated, with four replicates per treatment. Seeds were sown in sterilized, moist sphagnum moss in clear plastic trays (15 × 12.5 × 4.5 cm). Trays were randomly blocked in a germination chamber set at 30 °C with a 12-h daily photoperiod (80 μmol m^{-2} s^{-1}, cool white fluorescent light). Germination was recorded weekly until 1 wk after the last observed germination (week 7) and counted when the embryo dislodged the operculum. Due to fungal contamination in the sphagnum moss, all seeds were sprayed with a 1% solution of Captan 50-WP (ICI Americas Inc., Wilmington, DE) after 3 and 24 d of incubation.

Mean time to germination was calculated as MTG = $\sum (f x)/\sum f$; where f is the number of seeds germinated on day x and x is the number of days after sowing (Furutani *et al.* 1985). Per cent germination data were arcsine-transformed prior to performing analysis of variance on FGP and MTG using GLM in SAS version 9.1 (SAS Institute, Cary, NC). Post hoc mean separation was by Duncan's Multiple Range test at $\alpha = 0.05$.

Twelve adult rats (*R. rattus*) were captured from wild populations in mesic forest sites within the Wai'anae Mountains, O'ahu, transported to the University of Hawai'i Lyon Arboretum Rodent Housing Facility, and held in 38 cm × 22 cm × 18 cm metal-mesh cages (one rat per cage). Rats were allowed to acclimate for at least 1 wk before beginning feeding trials and between feeding trials, during which time they were fed a diet of mixed seeds (e.g. corn, sunflower, wheat, barley, oats, sorghum) and fruit wedges (tangerine). Rats were checked daily in order to ensure ample food and fresh water.

Two feeding trials took place, one for *P. hillebrandii* and one for *P. kaalae*. These were separated by 11 d and the same 12 rats were used for each trial. Prior to each trial, seeds were individually weighed and sealed in plastic bags. Three additional conspecific seeds were weighed and placed in bags which were opened and placed next to cages during the feeding trials to account for seed mass changes due to environmental hydration or dehydration. During each trial, one seed was placed in each cage and exposed
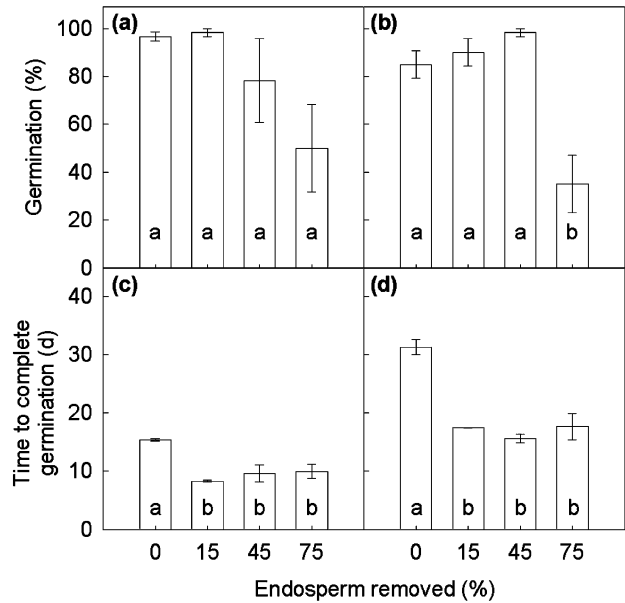

Figure 1. Germination of *Pritchardia hillebrandii* and *Pritchardia kaalae* seeds after removal of 0, 15, 45, or 75% of endosperm. Mean final germination after 49 d for *P. hillebrandii* (a) and *P. kaalae* (b). Mean number of days to complete germination for *P. hillebrandii* (c) and *P. kaalae* (d). Error bars denote SE, and N = 4 for each treatment. Columns within each panel with different letters are statistically significant at $\alpha = 0.05$ according to Duncan's Multiple Range test.

to a rat for 48 h. Seeds were inspected after 24 and 48 h, after which all control and trial seeds were collected and reweighed. Loss of seed mass was determined after accounting for change in mass of control seeds. If present, the condition of the embryo was also noted after rat exposure.

Mean FGP for *P. hillebrandii* and *P. kaalae* remained above 75% for up to 45% endosperm removal. Additionally, for both species, FGP in the minimally damaged (15%) and moderately damaged (45%) seeds did not differ significantly from undamaged seeds (Figure 1a, b). However, FGP dropped considerably when seeds were severely damaged; *P. hillebrandii* decreased to 50% ($F_{3,9} = 3.54$, P = 0.06; Figure 1a) and *P. kaalae* to 35% ($F_{3,9} = 54.9$, P < 0.0001; Figure 1b).

MTG for both species was greatest when endosperm remained intact (Figure 1c, d). *Pritchardia* seeds experiencing any level of simulated herbivory germinated approximately two times faster than control seeds (*P. hillebrandii*, $F_{3,9} = 9.84$, P = 0.003, and *P. kaalae*, $F_{3,9} = 25.3$, P = 0.0001). Additionally, germination at any level of endosperm removal tended to be more rapid for the smaller-seeded species (*P. hillebrandii*) than for the larger-seeded species (*P. kaalae*; Figure 1c, d).

Each of the 12 rats ate at least a portion of the offered seeds of both *Pritchardia* species, and on average > 80% of each seed was eaten (Table 1). With the exception of one

Table 1. Summary of *Rattus rattus* feeding trials. Each of the two species of *Pritchardia* seeds were individually offered to 12 rats (one seed per rat) for a 48-h period. The loss in seed mass (g) due to rats eating individual seeds was used to calculate the percentage of seed eaten. The total number of embryos remaining intact was out of 12 possible embryos for each *Pritchardia* species.

Species	Range of seed eaten (%)	Mean (± SD) of seed eaten (%)	Number of embryos remaining intact
P. hillebrandii	4.5–100	81.6 ± 25.4	1
P. kaalae	9.7–100	89.0 ± 25.7	2

rat that was offered *P. kaalae*, all seed damage occurred within 24 h for both species. In many cases, the entire seed was consumed (2/12 *P. hillebrandii* and 6/12 *P. kaalae*). Only one seed of each species was chewed < 45%, and few embryos remained intact (Table 1).

Most seeds germinated even when up to 45% of their endosperm was removed. Moreover, damaged seeds germinated faster than undamaged seeds. Our trials confirmed that *Pritchardia* seeds can suffer damage comparable to that which we simulated experimentally. Most *Pritchardia* seeds eaten by rats will die. However, the few seeds that are damaged by up to 45% may be affected by more rapid germination.

The ability to germinate when partially damaged may be due to the relatively large seed sizes of both species. Experimental and descriptive studies have found that large-seeded species retain the ability to germinate after approximately half of the seed mass was removed from *Quercus phellos* (Steele *et al.* 1993), four large-seeded tropical species from Papua New Guinea (Mack 1998), and two large-seeded neotropical species (Dalling & Harms 1999, Dalling *et al.* 1997). A greater abundance of nutritive reserves in larger seeds is the likely explanation for this pattern of germination by damaged seeds (Dalling *et al.* 1997, Mack 1998). While both *Pritchardia* species withstood removal of nearly half of their seed mass, more severe damage impaired germination. As demonstrated in the captive feeding trials, rats most often severely damaged (> 75%) *Pritchardia* seeds, suggesting that most seeds will be killed by rats and the few seeds that may survive will be those damaged < 45%.

The most rapid germination in this study occurred in damaged seeds. This result was contrary to our hypothesis that germination is delayed (i.e. MTG increases) with increasing damage. Rapid germination of *Pritchardia* seeds after damage suggests that structures covering the embryo may partly control germination. A thickened endocarp, seed coat, or dense endosperm can present a barrier to imbibition and germination (Pérez *et al.* 2008, Vallejo-Marín *et al.* 2006). Therefore, removal of at least 15% of the seed may facilitate rapid penetration of available moisture; hence decreased MTG. Accelerated

seed germination has been reported across a wide range of species experiencing seed damage levels of < 1% (Vallejo-Marín *et al.* 2006), < 25% (Koptur 1998), and 30–60% (Zhang & Maun 1991).

Damaged seeds can both positively or negatively influence recruitment (Côté *et al.* 2003, Janzen 1972, 1976; Mack 1998). Accelerated germination in partially damaged *Pritchardia* seeds may be beneficial for minimizing a seed's exposure to seed predators and pathogens. Damaged seeds can have elevated susceptibility to bacterial and fungal pathogens (Ceballos *et al.* 2002). Even if seed herbivory or pathogen infection is not immediately lethal, damaged seeds have less resources than non-damaged seeds and this may reduce seedling establishment or survival (Janzen 1976). It is plausible that damage to fruits and seeds during feeding, while not directly responsible for mortality, may still lead to recruitment failure by breaking seed dormancy when environmental conditions are not adequate for seedling establishment (Koptur 1998).

During captive feeding trials, rats damaged all *Pritchardia* seeds, most of them so severely they were killed. It is likely that rats interacting with these seeds in nature will typically destroy them. The few *Pritchardia* seeds that are damaged but not killed may experience accelerated germination, and if environmental conditions are favourable such seeds may have a competitive advantage over non-damaged seeds. Therefore, seed consumption does not always result in mortality, even in this example with tropical insular endemics that did not evolve with seed-eating mammals.

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