Interactions between rainforest trees and their vertebrate seed predators in continuous and fragmented habitat

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Abstract

Ground-active vertebrates can potentially exert a strong influence over the community composition of rainforest trees because they are significant predators of seeds. If the fragmentation of rainforest habitat alters the species composition of vertebrate seed predator assemblages, or patterns of interaction between seed predators and tree species, the subsequent patterns of tree recruitment are also likely to be affected. However, these interactions have not previously been investigated at a community-level. This thesis investigated patterns of community-level interactions between species of ground-active vertebrate seed predators and seeds from a range of common local rainforest tree species in the Big Scrub region of subtropical eastern Australia. In six sites in continuous forest and six sites in fragmented rainforest, ground-active vertebrates were surveyed using automated infra-red video cameras and predation on seeds of 20 tree species were assessed at experimental seed stations. In addition, laboratory analysis of the physical and chemical traits of the 20 rainforest tree species were conducted.

Camera surveys recorded 22 vertebrate taxa (12 birds, nine mammals and one reptile); 18 identified to species level and four to genus. Nine taxa were also identified as seed predators from the camera data, collectively exhibiting behaviours associated with potential or actual seed predation on 43% of camera days. A further seven of these were further defined as “common” seed predators, occurring in six or more sites, and analysed individually. The abundance and observed feeding behaviour of six taxa was significantly affected by fragmentation: two small mammals (Rattus fuscipes and Melomys cervinipes) suffered reduced abundance in fragments, while two small seed-eating birds (Chalcophaps indica and Leucosarcia melanoleuca), a large mammal (Trichosurus sp.) and a small mammal (Rattus rattus) increased in fragments, as did a suite of small native
ground-active insectivorous birds. For each taxon, levels of destructive interest in seeds generally increased in the habitat of increased abundance.

Data obtained from cameras were used with experimental seed stations to quantify the patterns of interactions between the seven common ground-active vertebrate taxa and the seeds of the 20 tree species. These interactions were found to diffuse and complex, with little evidence of specialised interactions. Seed predators were generalist in diet, consuming seeds from a wide range of tree species. Similarly, seeds of most tree species were consumed by a range of predators. The most important seed predator was *Rattus fuscipes*, which consumed seeds from more tree species than any other predator and showed the highest levels of destructive interest in seeds. Across all predators, no significant difference was found in levels of destructive interest in small (<10 mm) and large (≥10 mm) seeds, although the influence of seed size varied among taxa. Predator taxa were categorised as small birds, small mammals, large birds and large mammals (where small <1.0 kg, large >1.0 kg); large and small mammals collectively showed higher destructive interest in large seeds than small seeds. The level of destructive interest in seeds recorded by different functional groups correlated strongly with field damage by these groups to plasticine seeds placed together with the actual seeds of each species.

Relationships between the physical and chemical traits of the 20 seed species, and the field predation rates recorded at the experimental seed stations were investigated. Laboratory-based measurements of seed width, embryo/endosperm dry weight, fracture resistance, coat thickness, relative coat thickness and chemical antifeedant compounds were used to quantify seed traits. Seed size, coat thickness, fracture resistance and chemical properties all varied greatly among species. Overall, no strong predictions of field predation rates could be made based on measurements of seed traits, although seeds with higher fracture resistance tended to experience lower predation. Antifeedant
chemical compounds were obtained from 17 of the 20 study species. These belonged to four broad classes: alkaloids, terpenes, polyphenols and polyketides. Sixteen species contained cytotoxic compounds, one contained a compound with neurological effects and two contained compounds with both cytotoxic and antimicrobial properties, all of which were reported as possessing antifeedant properties from literature searches. A single class of antifeedant chemical compound was found in 16 of the 17 seed species identified with antifeedant compounds, while one species (Podocarpus elatus), contained two classes. Findings suggest that rainforest tree species use a range of different combinations of seed traits as a proximate defence against vertebrate seed predators.

The rates of seed predation at experimental seed stations were compared between continuous forest and fragments. As a measurement of seed predation, an ‘interference score’ was calculated from the numbers of seeds removed or damaged. Overall, 46% of seeds were removed from seed stations after five days exposure, also giving an overall average interference score of 0.49. Interference scores varied greatly among seed species (0.20 to 0.77). A strong statistical interaction between habitat type (fragment and continuous) and species of seed showed that the effects of habitat fragmentation on seed predation also varied greatly among species. Five individual species experienced significantly greater predation in fragments (Acmena hemilampra, Castanospora alphandii, Mallotus philippensis, Melia azedarach and Wilkea huegeliana), and five species significantly less (Brachychiton acerifolius, Castanospermum australe, Cinnamomum camphora, Cryptocarya obovata and Tabernaemontana pandacaqui) than in continuous forest, although the effects of fragmentation on rates of predation were not related to seed size.

These findings show that rainforest fragmentation has significantly altered the species composition of vertebrate seed predators in rainforests of the Big Scrub. Habitat
fragmentation has also substantially affected the complex community-level interactions between individual seed predators and seeds. The richness of interactions between individual predator taxa and seed species was higher in fragments than in continuous forest; the most important predator in continuous forest (*R. fuscipes*) was less common in fragments while several other predators (two native birds, *C. indica* and *L. melanoleuca*, one large possum, *Trichosurus* sp. and the introduced rodent *R. rattus*) were more common, and also interacted with a greater number of seed species. These altered rates of seed predation in fragments are likely to lead to shifts in patterns of plant recruitment.

The benign effects of fragmentation on populations of native bird species indicate that well-managed rainforest fragments can support a diverse community of ground-active vertebrate seed predators. Management options focusing on protecting and maintaining the habitat quality of existing fragments, as well as increasing connectivity in the surrounding landscape matrix could enable existing populations of native ground-active birds to maintain their populations. These management actions could also sustain populations of native mammals that would otherwise be significantly reduced in habitat fragments, and thereby produce patterns of seed predation and plant recruitment which resemble those observed in continuous forest.
Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)_____________________________
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Journal articles arising from this thesis

Chapters 2, 3, 4 and 5 in this thesis are co-authored manuscripts. My contribution to each co-authored paper is outlined at the front of the relevant chapter. Appropriate acknowledgements of those who contributed to the research but did not qualify as authors are included in the Acknowledgements section of this thesis. Chapters 2, 3, 4 and 5 have been prepared for publication. The bibliographic details for these papers are:

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Chapter One

General introduction

1.1 Plant-animal interactions and their influence on plant recruitment in rainforests

Plants and animals in rainforest communities are involved in complex and diverse ecological interactions (Jordano et al., 2003; Link and Di Fiore, 2006; Ollerton et al., 2011). Many of these interactions influence the potential of plants to regenerate. For example, many plant species in rainforests rely on animal-mediated pollination, mostly by insects (Bawa, 1990; Hegland and Totland, 2008), but also by bats and birds (Dick et al., 2003; Lunau et al., 2011). Rainforest plants also rely heavily on frugivorous animals to disperse their seeds: 70% - 90% of plant species in rainforests produce fleshy fruits (Jordano, 2000; Sridhara et al., 2016). Once the seeds of rainforest plants have been deposited on the forest floor, they may then be consumed by vertebrate seed predators, resulting in high rates of seed mortality: often above 50% within 30 days of being deposited on the forest floor (DeMattia et al., 2004; Donatti et al., 2009). Thus, any factors which influence the rates of post-dispersal vertebrate seed predation could strongly influence the probability of seeds’ surviving to germination and becoming seedlings (Wenny; 2000; Paine and Beck, 2007).

Most research into the effects of post-dispersal seed predation by rainforest vertebrates has occurred in the neotropics, where a wide range of mammals have been identified as vertebrate seed predators. For example, large-bodied mammals weighing up to 40 kg, particularly peccaries (family Tayassuidae) (Demattia and Rathcke, 2004), large-bodied rodents greater than 1 kg, including agoutis (family Dasyproctidae) (Silvius and Fragoso, 2003) and a variety of small-bodied rodent species within families such as
Cricetidae and Heteromyidae have been identified as seed predators (Sánchez-Cordero and Martinez-Gallardo, 1998; Iob and Vieira, 2008).

Previous research has provided evidence that changes in the species composition of mammals, in particular of rodent species, can lead to significant changes in rates of post-dispersal seed predation in rainforests. For example, in the neotropics, the white-lipped peccary (*Tayassu pecari*), a large mammalian seed predator of the palm species *Euterpe edulis* has gone locally extinct as a result of habitat loss and poaching. Subsequently, predation on this plant species by several species of small (<1000 g) and large (2000-4000 g) rodents (families Dasyproctidae and Muridae) increased, resulting in higher levels of seed destruction (Galetti et al., 2015). Depleted populations of rodents ranging in size from 200-4000 g (Dasyproctidae and Sciuridae) in forests affected by poaching, and lower rates of seed predation on the palm species *Attalea butyracea* have also been reported in the neotropics (Wright and Duber, 2001).

Such changes in patterns of vertebrate seed predation can ultimately lead to altered patterns of plant recruitment. For example, Wright et al. (2007) found that the average mass of seeds surviving to germination was greater in forests unprotected from hunters, who had been targeting large (>1000 g) seed predators (*Dasyprocta agouti* and *Tinamus major*) than in protected forest; this resulted in shifts in plant species composition between protected and unprotected sites. Post-dispersal seed predation by vertebrates can thus have a longer term influence over plant species composition in rainforests (Wright et al., 2007; Effiom et al., 2013).

In spite of cases such as these, which have studied specific interactions between certain vertebrates and the seeds of particular trees, the species composition of vertebrate seed predator assemblages remains poorly known. Furthermore, community-level interactions between the range of coexisting seed predator species and seed species have
not been investigated. Although there is a lack of research investigating seed-seed predator interactions, studies focusing on plant-frugivore relationships have reported complex networks of interactions, with low levels of specialization between species-specific pairs of plants and frugivores (Bluthgen et al., 2007; Schluening et al., 2011). In further research focusing on these interactions, morphological traits of frugivores have been identified as predictors of the types of fruits that may be consumed by frugivores. For example, Moran and Catterall (2010) reported that birds with larger gape widths commonly consumed larger fruits.

Rainforest trees produce seeds of an extremely wide variety of sizes, ranging from seeds of the family Orchidaceae (width, 0.2 mm; weight, $1 \times 10^{-6}$ g) to seeds of *Lodoicea maldivica* (width, 30 cm; weight, 20 kg) (Harper et al., 1970). Many seeds of rainforest trees are also physically well-protected by thick seed coats. For example, Blate et al. (1998) recorded coat thickness across 40 tree species, ranging from 0.1-4.6 mm. It is possible that morphological traits of seed predators may restrict the seed species they are able to consume, thus influencing the preferences of a given predator species for particular seed species over others. Chemical defence may also protect seeds from predation: the vegetative parts of plants have been widely reported as containing many secondary compounds toxic to animals (Spalinger et al., 2010; Mithofer and Boland, 2012). While much less is known about the chemical properties of seeds, some secondary compounds in seeds have been reported as providing protection against attack from insects (Cardinal-Aucoin et al., 2009) and pathogens (Mohamed-Yasseen et al., 1994), and it is possible that secondary compounds in seeds may also act as defence against vertebrate attack. A better understanding of how the functional traits of seed species are related to their patterns of consumption by different vertebrate species could provide greater insights into community-level interactions between rainforest plants (particularly trees) and their post-dispersal predators.
1.2 Rainforest clearing and fragmentation

Tropical rainforests support the majority of the world’s biodiversity (Gibson et al., 2011; Hernández-Ruedas et al., 2014), however some 35-50% of these rainforests had been cleared by 2004 (Hansen and DeFries, 2004), while it is estimated that approximately 5.8 million hectares of tropical forest are cleared each year (Achard et al., 2002). Habitat loss associated with deforestation can result in depleted populations or localized extinction of many species of rainforest dependent plants and animals (Brook et al., 2003; Laurance and Peres, 2006). Deforestation also results in habitat fragmentation, where formerly large areas of continuous forest are reduced to isolated fragments, scattered across highly modified landscapes. The increased isolation of rainforest fragments can lead to significant changes in species composition of both flora and fauna (Gerber et al., 2012; Cararra et al., 2015).

Rainforest vertebrates can be particularly vulnerable to reduced abundance in fragmented habitat. For example, larger-bodied vertebrates with larger area requirements tend to be at greater risk of reduced abundance or extinction in fragments than smaller-bodied vertebrates (Chiarello, 1999; Cardillo and Bromham, 2001; Vetter et al., 2011), however the effects of fragmentation can be complex and highly variable: body size, population density, tolerance to the matrix surrounding fragments, degree of habitat specialization and dispersal abilities can all influence a species ability to persist in fragments (Bennett and Owens, 1997; Lees and Peres, 2009; Salisbury et al., 2012). Consequently, many processes are involved in producing vertebrate communities in rainforest fragments that are likely to differ in many ways from those in large, uncleared forest tracts.

These changes in rainforest vertebrate communities are likely to have secondary effects on the range of plant-animal interactions (such as pollination, seed dispersal and
seed predation) that play important roles in the reproduction and recruitment of rainforest plants, and consequently in the community dynamics of rainforest trees (Moran et al., 2004; Garcia and Chacoff, 2007; Kolb, 2008). Studies of how the abundance of ground-active vertebrate seed predators and their rates of seed predation are influenced by rainforest fragmentation have targeted particular guilds or size classes of seed predators, and focused on one or a few species of seeds, producing contrasting results. For example, in neotropical rainforest, depleted populations of rodents (family Cricetidae) in fragments led to reduced predation on one tree (Cabralea caijerana) species (Pizo, 1997), while Guariguata et al. (2002) reported that reduced abundance of larger (> 30 kg) mammalian carnivores (including Panthera onca, Leopardus pardalis) may have released small (<1000 g) rodents (family Heteromyidae) from competition, resulting in increased rodent predation on two tree species (Carapa pianensis and Dipteryx panamensis) in fragments. Fragmentation may also differentially affect rates of predation based on seed size. For example, a reduction in abundance of large-bodied (>1000 g) seed predators with greater potential to consume large (> 100 mg) seeds may favor large–seeded tree species by reducing rates of predation on them (Wright et al., 2007). Furthermore, in a fragmented central American rainforest predation by small (<1000 g) rodents was higher on small (15 mm) seeds than on large (30 mm) seeds where populations of larger-bodied (>7 kg) mammals had been depleted (Dirzo and Mendoza, 2007). At the whole-community level, the effect of rainforest fragmentation on multiple coexisting species of ground-active vertebrate seed predators and their interactions with multiple rainforest tree species have not previously been studied.

1.3 Australian rainforests and their fauna

Rainforests once covered much of the Australian continent, but a shift over millions of years from a climate that was warm and moist to cooler and drier conditions
resulted in their contraction and subsequent restriction to discontinuous refugial areas with suitable climatic conditions (Adam, 1994). As a result, contemporary Australian rainforests are largely restricted to the east coast, with tropical rainforest occurring in the continents northeast and subtropical rainforests in mid-eastern latitudes (Bowman, 2000).

It has been estimated that Australian rainforests contain approximately half of Australia’s indigenous biodiversity (Adam, 1994). However, approximately 70% of lowland rainforest in tropical Australia was cleared by European settlers in the nineteenth and twentieth centuries, mostly for agricultural development (Winter, 1976), with approximately 60% of subtropical rainforests also cleared (McDonald et al., 1998; Kanowski et al., 2003). Many areas of remaining Australian rainforests presently exist as a series of isolated fragments scattered across highly modified landscapes (Adam, 1994).

With respect to subsequent impacts on vertebrate communities, Harrington et al. (2001) reported a substantially altered species composition between continuous forest and fragments in assemblages of nine small ground-active mammals of the Australian wet tropics. Small mammals capable of tolerating modified landscapes have been able to persist in fragments (Laurance, 1994), while among arboreal rainforest mammals, a species’ ability to disperse across modified habitats (for example making use of corridors of secondary growth) has been considered a predictor of whether a species can persist in fragments (Pahl et al., 1988; Laurance, 1990). For rainforest bird communities in the wet tropics, Warburton (1997) concluded that generalist species that were more able to persist in the surrounding matrix tended to be numerically dominant in the smallest fragments.

In the subtropics, Bentley et al. (2000) reported variable responses of small ground-active mammals to forest fragmentation, with habitat generalists being largely tolerant of fragmentation and habitat specialists vulnerable. Further south, in warm-temperate rainforest, Dunstan and Fox (1996) found similarly variable patterns among
small ground-active mammals, with two species decreasing in abundance, and one increasing in highly disturbed fragments. Among frugivorous birds in subtropical rainforest, Moran (2007) found that some species decreased in abundance in fragments while some species increased and others were unaffected.

There has been little research into how the fragmentation of Australian rainforests affects plant-animal interactions. Moran et al. (2004) found that the frugivorous bird species which had highest potential to disperse seeds of large-seeded (width, ≥10 mm) plant species were less abundant outside of intact rainforest, potentially limiting their dispersal. In the wet tropics, the rodents Uromys caudimaculaius, Melomys cervinipes, Rattus fuscipes have been identified as seed predators (Osunkoya, 1994; Harrington et al., 1997), while Harrington et al. (1997) reported no significant effect of fragmentation on the abundance of U. caudimaculaius, or on its rates of predation on three tree species.

The ‘Big Scrub’ is a region that, prior to European settlement of Australia, contained some 750 km² of subtropical lowland rainforest (Floyd, 1990). The Big Scrub was almost completely cleared in the late nineteenth century, reducing the remaining rainforest to a series of isolated fragments totalling less than 1% of its original area (Floyd, 1990; Lott and Duggin, 1993). In the Big Scrub, small-mammal trapping surveys have found substantial differences in communities of ground-active rodents between fragments and continuous forest (Lott, 1997; Stephen, 1999; Fine, 2005), together with reduced seed predation on a common tree species, the black bean Castanospermum australe (Lott, 1997; Fine, 2005). However, neither the full range of ground-active vertebrate seed predators (bird and mammal), nor their range of responses to fragmentation have been studied. Furthermore, the patterns of seed predation by these vertebrates across a range of different locally common rainforest tree species are also
unknown, although the limited previous research into *C. australis* indicates the potential for these patterns to be affected by fragmentation.

1.4 Aims and structure of thesis

The aim of this thesis is to investigate the pattern of community-level interactions between species of ground-active vertebrate seed predators and a range of common local rainforest tree species, and to identify how these interactions are affected by habitat fragmentation. Specifically, these ecological processes are investigated through a combination of camera-based field surveys of ground-active vertebrates and experiments investigating rates of seed predation in continuous and fragmented rainforest in the Big Scrub region of Australia’s subtropics; together with laboratory analysis of the physical and chemical traits of 20 local rainforest tree species.

In Chapter 2, the composition and relative abundance of vertebrate seed predator species in fragments and continuous forest are identified and compared. The relative amounts of destructive interest in seeds shown by different seed predator species are also assessed and compared between fragments and continuous forest.

Chapter 3 investigates the patterns of community-level interaction between the seeds of 20 locally common tree species and the common individual seed predator species, by quantifying levels of destructive interest shown by each seed predator in each seed species.

Chapter 4 investigates the extent to which the physical and chemical traits of seeds of the same 20 tree species influence their rates of seed predation by vertebrates. Specifically, physical defence, chemical defence and nutritional value of seeds are assessed with the aim of establishing if any of these traits can predict levels of predation.
Chapter 5 investigates how the predation rates by ground-active vertebrates on these 20 seed species are affected by habitat fragmentation, including the effects of seed size, seed species and their interaction with fragmentation.

Chapter 6 presents a summary of the findings of this thesis, and discusses the effects of fragmentation on the composition of ground-active vertebrate seed predators and the subsequent changes in patterns of interaction between these vertebrates and seeds. The likely changes in tree species composition of fragments, and ecological and management implications for the rainforests of the Big Scrub are also discussed.
Chapter Two

How does rainforest fragmentation affect the species composition of ground-active vertebrates and their patterns of seed consumption?

Abstract

Rainforest fragmentation can have significant impacts on species composition of vertebrate assemblages, and post-dispersal seed predation by rainforest vertebrates can be an important driver of plant community composition. Therefore, any changes to vertebrate assemblages as a result of fragmentation may have a significant impact on patterns of plant recruitment. Using automated infra-red cameras, this study investigated the species composition and abundance of vertebrate post-dispersal seed predators, and their interactions with seeds of locally occurring tree species in continuous and fragmented subtropical rainforest of eastern Australia. Deployment of infra-red camera traps with and without seeds provided records of 18 taxa (mainly birds and mammals) identified to species and a further four to genus; nine of which were identified as seed predators. Collectively, all nine predator taxa exhibited behaviours associated with potential or actual seed predation on 43% of camera days. *Rattus fuscipes* was the most abundant seed predator and also showed the highest levels of destructive interest in seeds. Six individually analysed predator taxa were significantly affected by fragmentation: two small mammals (*R. fuscipes* and *Melomys cervinipes*) suffered reduced abundance in fragments, while two small seed-eating birds (*Chalcophaps indica* and *Leucosarcia melanoleuca*), a large mammal (*Trichosurus* sp.) and a small mammal (*Rattus rattus*) increased in fragments. Levels of destructive interest in seeds were also significantly affected by fragmentation, generally increasing in the habitat of increased abundance. Rainforest fragmentation substantially altered both the species composition of vertebrate
seed predators and their behavioural interactions with seeds. These changes are likely to result in altered patterns of plant recruitment following rainforest fragmentation.

2.1 Introduction

Rainforest fragmentation can lead to significant changes to vertebrate species assemblages (Ewers and Didham, 2005; Bregman et al., 2014). Research in neotropical rainforests has indicated large-bodied vertebrate species are at greater risk of reduced abundance in forest fragments than small-bodied species (Chiarello, 1999; Gilbert and Setz, 2001; Melo et al., 2010). However, fragmentation effects on vertebrates can be complex and variable. For example, in South American rain forest, Fleury and Galetti (2006) reported reduced abundance of small (<1000 g) squirrel (Sciurus ingram) populations in fragments <100 ha, concluding that this species was unable to survive in less dense vegetation associated with small fragments. On the other hand, the abundance of small mammals capable of traversing disturbed or modified habitats may increase in fragments. For example, in the Australian tropics Laurance (1994) found an increase in abundance of four common small (<1000 g) mammal species (families Dasyuridae, Muridae) tolerant of modified matrices surrounding fragments while those that avoided modified habitat declined in abundance or disappeared.

Vertebrate seed predation in rainforests can lead to high levels of seed mortality, for example in South America, Fleury et al. (2014) recorded seed mortality by rodents on four species at 70% after 30 days. Consequently, changes in vertebrate seed predator assemblages may have significant effects on rates of seed predation and subsequent patterns of plant recruitment. Galetti at al. (2015) found that poaching and fragmentation led to the local extinction of a large (approx. 30 kg) seed predator, the white-lipped peccary (Tayassu pecari) from South American rainforests, resulting in substantial overcompensation in rates of seed predation by several species of rodents (families
Muridae and Dasyproctidae) on one palm species (*Euterpe edulis*). However this study could not determine if this was due to an increase in rodent abundance as a result of reduced competition with large herbivores or an increase in rodent activity. Also in South America, Klinger and Rejmanek (2013) experimentally reduced the density of a seed-eating rodent (*Heteromys desmarestianus*), finding rates of seed predation decreased and germination increased on nine tree species.

The majority of studies investigating seed predators in rainforests have occurred in the neotropics, where rodents are considered to be important seed predators (Forget, 1996; Forget, et al. 1999; Iob and Vieira, 2008). These studies have used seed feeding trials (Munoz and Bonal, 2008) or exclosure experiments designed to exclude vertebrates of particular sizes, and thereby to identify potential seed predators (Farwig, et al. 2008). However, these methods are labour intensive and time consuming. Furthermore, they have tended to focus on mammals as potential seed predators, while birds have rarely been targeted (but see Lasso and Ackerman, 2004). The recent availability of automated infra-red camera recorders, which can detect the presence of vertebrates (Silveira et al., 2003) and directly record their activities, now provides the opportunity to overcome these limitations. However they have rarely been used in published studies of post-dispersal predation of rainforest seeds.

The subtropical rainforests of Australia have been extensively cleared since European settlement, with existing remnants scattered across a highly modified landscape (Adam, 1994). Previous studies have found that mammal populations can be significantly affected by fragmentation of Australian subtropical rainforests. For example, Bentley et al. (2000) reported that responses of small ground-dwelling mammals in subtropical rainforest varied, with habitat generalists largely tolerant of fragmentation and habitat specialists vulnerable. Native rodents such as *Rattus fuscipes* and *Melomys cervinipes*
have been particularly vulnerable to reduced abundance in fragments, while abundance of the introduced *Rattus rattus* has increased in fragments (Lott 1997; Stephen, 1999; Bentley et al., 2000; Fine, 2005). In warm-temperate Australian rainforest, Dunstan and Fox (1996) found similar patterns, with the abundance of two native small mammals, *R. fuscipes* and *Antechinus stuartii* decreasing in abundance with decreased remnant size, and increased numbers of *R. rattus* in highly disturbed fragments.

While it has been well established that mammals are functionally important as seed predators in rainforests (Garcia et al., 2005; Paine and Beck, 2007; Donatti et al., 2009; Fleury et al. 2014) at the level of an ecological community, the species composition of vertebrate seed predator assemblages remains poorly known. The effects of rainforest fragmentation across a vertebrate seed predator community have not been studied in any region. This study uses infra-red camera technology to identify the composition and relative importance of seed-eating vertebrates on the rainforest floor in the ‘Big Scrub’ region of subtropical eastern Australia, and investigates the rates of interactions between seed predator species and seeds, and assesses how species composition and interactions with seeds have been affected by habitat fragmentation.
2.2 Methods

2.2.1 Study region and site network

In the “Big Scrub” region of eastern Australia (28°35'-28°48'S, 153°10'-153°31'E), the temperature ranges from 13˚C (mean annual minimum) to 26˚C (mean annual maximum) and rainfall ranges from 1343 mm to 2327 mm, with a distinct wet season occurring from November to May (Bureau of Meteorology 2005). The region’s vegetation has been characterised as warm subtropical rainforest by Floyd (1990) and is characterised by a high diversity of plant species and tall canopy trees that form a closed but uneven canopy. Agricultural development of the Big Scrub region resulted in significant land clearing between the late 1800’s and the late 1900’s, and the remaining areas of original rainforest are highly fragmented, estimated to be 300-556 ha in total area (Floyd, 1990; Lott and Duggin, 1993), or 0.4-0.7% of the original 75,000 ha. The largest remnants (75-150 ha) are located at the Big Scrub’s northern end, where they are contiguous with wet sclerophyll forests and upland rainforests. Other remnants range in size to a maximum of about 20 ha (Lott & Duggin, 1993; Lott, 1997; Parkes et al., 2012) and are scattered throughout the modified agricultural landscape. A large part of the landscape at the time of this study was livestock pasture, together with substantial areas of macadamia tree plantations, as well as woody forest regrowth, the latter two having developed since the late 1900’s (Parkes et al., 2012).

Twelve study sites spread throughout the region were selected for the present study: six in continuous forest (75-150 ha) and six in fragments (4-21 ha), all on lowland basaltic soils between 100 and 200 m altitude (Figure 2.1, see also Figure S2.1.1). Each study site covered an area of approximately 100 m x 100 m (10,000 m²). Four of the continuous sites were located in the Big Scrub Flora Reserve at the region’s northern end, and were separated by minimum distances of 500 m. The remaining two continuous sites
(Boomerang Falls and Minyon Falls), were also in the north, separated from each other by approximately 4 km (Figure 2.1). Sites in forest fragments were separated by between 4 km and 10 km, were located within the cleared agricultural landscape, ranged in size from 3 to 21 ha, and were 6.5-29 km distant from the continuous rainforest areas to the north. Sites were selected based on a previous regional assessment of environmental characteristics and floristic surveys (S. Mckenna, C.P. Catterall and J. Kanowski, unpublished data) in order to standardise soil type, elevation and vegetation type among sites.

Figure 2.1 Study site locations and major water courses (solid lines). Triangles represent continuous sites, squares represent fragments. Dashed line indicates boundary of Big Scrub rainforest prior to European settlement (Floyd 1990).
2.2.2 Field data collection

To investigate seed predation by vertebrates, seed stations were established at each site, using the seeds of 20 tree species known from floristic surveys to occur commonly in the study sites; nine were large-seeded (≥ 10mm) and 11 small-seeded (< 10mm). A seed station for a given species was established by tethering five seeds, together with an additional similar-sized “seed” constructed from brown plasticine to a central metal peg, with 50 cm of nylon fishing line (Figure S2.1.2). Plasticine seeds were used as part of an associated study investigating patterns of interactions between seeds and seed predators in the same study sites. Two stations for each species were established in each site, totalling 44 stations per site (18 species were used once and two were used twice). Seed stations were at least 50 m apart. Each station was left in place for five days, after which the fate of seeds was recorded as: “intact” (if no detectable evidence of any physical interference), “damaged” (when there was evidence of physical interference, such as teeth marks on the seed coat or partial removal of endosperm or embryo), or “removed” (if completely absent).

To record the vertebrate seed predators and their behaviour, one automated motion-activated infra-red video camera was focused on the seeds at one station for each seed species at each site (i.e. a total of 22 camera sessions with seeds at each site, Figure S2.1.3). Additionally, six camera traps were established at stations without seeds at each site, giving an overall total of 28 five-day camera sessions at each site (140 camera days, comprising 110 days at stations with seeds and 30 days at stations without seeds). Ltl Acorn 5210A 12MP trail hunting scouting cameras and Scout Guard TC2201NA cameras were used. Most events that activated the cameras’ motion sensors were movements of vertebrate animals.
These observations took place across two field seasons; in September-December 2013 and June-August 2015. Ten seed species (Acmena ingens, Castanospora alphandi, Cryptocarya glauescens, Cryptocarya obovata, Ehretia acuminata, Guioa semiglaucia, Mallotus philippensis, Neolitsea dealbata Tabernaemontana pandacaqui and Wilkiea huegeliana) were used in the first season, and twelve seed species (Acmena hemilampra, Atractocarpus chartaceus, Brachychiton acerifolius, Castanospermum australe, Cinnamomum camphora, Cryptocarya glauescens, Cryptocarya microneura, Denhamia celastroides, Ehretia acuminata, Eupomatia laurina, Melia azedarach and Podocarpus elatus) in the second season.

Where possible, all recorded vertebrates were identified to species when footage was viewed; in other cases identification was to genus level. To investigate behavioural interactions with seeds, three categories of interest in seeds were defined for each footage sequence in which an individual animal was recorded at a seed station. “No interest” was defined as an individual showing no physical response to seeds; “non-destructive interest” as an individual showing interest in seeds but not physically interfering with them; and “destructive interest” as an individual physically interfering with seeds.

2.2.3 Data summary and analysis

In total, vertebrates were recorded on 56.2% of the 1680 camera days, and 22 vertebrate taxa were identified, 18 to species and four to genus. The latter were subsequently attributed to most likely species based on knowledge of the region’s rainforest fauna. Fifteen taxa occurred in six or more sites and were defined as “common”.

To provide an index of vertebrate abundance at each site, I calculated the percentage of total camera days (140/site) during which each vertebrate taxon was present, henceforth termed its “occupancy rate”; these values were then averaged across all 12 sites. To assess whether the presence of seeds influenced the relative abundance of
vertebrates, I calculated the relative abundance of each taxon from cameras with and without seeds: the percentage of camera days with seeds on which a given taxon was recorded (of 110 camera days/site), as well as the percentage of camera days without seeds on which it was recorded (of 30 camera days/site); these values were then also averaged across the 12 sites. For each taxon, the value without seeds was subtracted from the value with seeds to generate a “presence interest” score; larger values indicate that the animal tended to spend more time near cameras if seeds were also present.

To quantify behavioural interactions of each vertebrate taxon with seeds, I calculated the percentages of days in which a given taxon showed either destructive interest or non-destructive interest in seeds at each site; in both cases using the total number of camera days on which it was recorded as the denominator. These values were then averaged across the number of sites where recorded (usually 12), to give both a “destructive interest” score and a “non-destructive interest” score for each vertebrate taxon.

Each taxon was classified as a seed predator or otherwise using both the literature and the field data. A bird was considered to be a potential seed predator if it consumed seeds as part of its primary diet according to the description in the HANZAB series (Higgins and Davies, 1990; Higgins and Peter, 1990; Higgins et al., 1990; Marchant and Higgins, 1990). Potential mammalian seed predators were identified using information in Strahan (1995) and for the single recorded reptile species, information in Guarino (2001) was used. For all species recorded more than once, destructive interest scores >0.10 corresponded with the literature-based assessment of potential seed predator role, and destructive interest scores of zero corresponded with seeds not being part of the primary diet. Seven taxa thus identified as seed predators were recorded in six or more of this study’s sites, and these were defined for further analysis as “common seed predators”.

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The following functional groups were also analysed: small birds, small mammals, large birds and large mammals (where small and large refer respectively to body weights below or above 1.0 kg); these groups included all predator taxa irrespective of their frequency.

For a given site, independent measurements of physical rates of seed predation were also obtained from the data on each seed’s physical fate at the end of the five-day exposure period. At each site, the percentage of the 12 seeds (actual or plasticine) that were either removed or damaged (cameras present or absent) was calculated across all species, large-seeded species and small-seeded species.

Correlation analysis using taxa as replicates was used to assess levels of interest in seeds irrespective of known diet, comparing the occupancy rates (across all sites) with versus without seeds (N=15 common taxa, occurring at six or more sites). This was supplemented by a paired t-test using the 12 sites as replicates, for all common taxa combined, of percent presence with versus without seeds. Behavioural responses to seeds were further tested using correlation analysis of scores for non-destructive interest versus destructive interest using the seven common seed predator taxa as replicates.

The effect of forest fragmentation on the site occupancy rates of the seven common seed predator taxa and functional groups was assessed using t-tests comparing fragments and continuous forest (N=6 sites in each), or Mann-Whitney U tests if data did not meet assumptions for t-tests. The pattern of variation in composition of common seed predator taxa among all 12 sites was visualised using two-dimensional nonmetric multidimensional scaling (MDS) ordination, with Bray-Curtis between site dissimilarities, using the ‘Vegan’ package, version 2.3-5, with 4,999 iterations in RStudio version 3.1.2 (Oksanen et al., 2015). Biplot vectors were added to the ordination plot to display individual taxa that were significantly (P<0.05) associated with the ordination pattern. To test the statistical significance of differences in seed predator composition
between sites in fragments and continuous forest, analysis of similarities (ANOSIM) was used, also in the Vegan package.

The effects of forest fragmentation on each taxon’s destructive interest score was tested using t-tests comparing fragments and continuous forest (N=6 sites for each). Finally, the physical damage or removal of seeds recorded at all stations (with and without cameras) after five days exposure was compared with the camera-based behavioural evidence of seed predation using correlation analysis, using the 20 seed species as replicates, to compare the percent of seeds that were physically damaged or removed with the total camera days in which destructive interest was recorded. The percentage of seeds physically damaged or removed across all seed species was also compared between sites in fragments and continuous forest (N=6 in each) using t-tests, and similar tests were done for small- and large-seeded species separately.

2.3 Results

2.3.1 Seed predator community composition

Across all sites, 22 vertebrate taxa were recorded, comprising nine mammals, 12 ground dwelling birds and one reptile (Table 2.1, see also Table S2.2.1). Occupancy rates across taxa varied greatly, from the large bird *Accipiter novaehollandiae* (BACNO), recorded on one day in one site only, to the large mammal *Thylogale* sp. (MTHSP), recorded on 27.3% of days across all 12 sites.

Seven taxa were identified as common seed predators (present at six or more sites). These comprised three small mammal rodents (*Rattus fuscipes* (MRAFU), *Rattus rattus* (MRARA), *Melomys cervinipes* (MMECE)), one large marsupial mammal (the possum *Trichosurus* sp. (MTHSP)), one large bird (*Alectura lathami* (BALLA)), and two small birds (*Chalcophaps indica* (BCHIN)) and *Leucosarcia melanoleuca* (BLEME)).
Occupancy rates of seed predator taxa varied from *M. cervinipes* (MECE), recorded on 1.3% of days to *R. fuscipes* (MRAFU), recorded on 20.7% of days (Table 2.1). *M. cervinipes* (MMECE) was recorded in six continuous forest sites, and all other common predators were recorded in all 12 study sites.

2.3.2 Behavioural responses to seeds

Across all 15 common taxa, there was a strong association between the relative abundance with versus without seeds (*t*=0.14, *r*=0.73, *P*=0.0003, *N*=15; Figure 2.2). One species, *R. fuscipes* (MRAFU), exhibited an unusually high “presence interest” response to seeds (Table 2.1, Figure 2.2), although when *R. fuscipes* (MRAFU) was excluded the relationship remained strong (*t*=0.26, *r*=0.89, *P*=0.0001, *N*=14; Figure 2.2). To assess whether the data from camera traps provided a good indication of relative abundance, I tested whether the slopes of the regression lines differed significantly from 1.0. The slope did not differ significantly from 1.0 for all 15 common taxa (*b*=0.84, *t*=1.11, *P*=0.34, *N*=15), nor when *R. fuscipes* was removed from analysis (*b*=1.11, *t*=1.00, *P*=0.89, *N*=14). However, all common taxa collectively were slightly more likely to be recorded by cameras with seeds than cameras without seeds (paired t-test, *P*=0.04, *N*=12 sites). Removing *R. fuscipes* (RAFU) from analysis reduced the effect of seed presence on the relative abundance of vertebrates (paired t-test, *P*=0.06, *N*=12 sites).
Table 2.1 Vertebrate taxa recorded from camera traps, and their frequencies of occurrence and behavioural responses to seeds. ‘Group’ refers to the functional group of each taxon: SB, small bird; LB, large bird; SM, small mammal; LM, large mammal; R, reptile. ‘Diet’ is the primary diet of each taxon: S, seeds; F, fruit; P, vegetative plant parts; I, invertebrates; V, vertebrates; numbers indicate the sources of information on primary diet: 1, Guarino 2001; 2, Higgins and Davies 1996; 3, Higgins and Peter 2002; 4, Higgins et al. 2001; 5, Marchant and Higgins 1993; 6, Strahan 1995. ‘No. sites’ is the number of sites in which a taxon was recorded. ‘Pred’ indicates whether or not a given taxon was classified as a seed predator (Y, yes; N, no).

<table>
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<tr>
<th>Group</th>
<th>Abbreviation</th>
<th>Taxon</th>
<th>Diet</th>
<th>No. Sites</th>
<th>% days present</th>
<th>PF*</th>
<th>Non-DI</th>
<th>DI*</th>
<th>Pred</th>
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<tr>
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<td>Rattus ratten</td>
<td>SFPI; 6</td>
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<td>7.1</td>
<td>0.18</td>
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<td>Tachyglossus aculeatus</td>
<td>I; 6</td>
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<td>0.2</td>
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Seed predator functional groups

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<td>10.4</td>
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<tr>
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<td>1.1</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>B</td>
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<td>14.6</td>
<td>0.05</td>
<td>0.33</td>
</tr>
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<td>21.8</td>
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<td>0.41</td>
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[^1]: % days present=average percentage of days a given taxon was recorded by cameras across 12 sites
[^2]: PI=“presence interest”; average percentage of days in which a given taxon was recorded at cameras without seeds, subtracted from the equivalent value for cameras with seeds
[^3]: Non-DI=“non-destructive interest”; average (across sites) percentage of days in which a given taxon was recorded showing interest in seeds but not physically interfering with them, calculated from the total number of days in which it was recorded
[^4]: DI=“destructive interest”; average (across sites) percentage of days in which a given taxon was recorded behaviourally interfering with seeds, calculated from the total number of days in which it was recorded

5. *M. cervinipes* is the only known *Melomys* species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005)
6. Most likely *T. thetis* however may potentially include *T. stigmaticus*
7. Most likely *T. caninus* or *T. vulpecula*
**Figure 2.2** Effects of seeds on camera detection of all vertebrates. Points represent the 15 common taxa (see Table 1 for abbreviations). Italics show taxa not classified as seed predators. Axes show the percent of camera days (averaged across 12 sites) in which each vertebrate taxon was recorded, either with or without seeds present. The solid line shows the linear relationship when all taxa are included. Dashed line excludes MRAFU.

Levels of non-destructive and destructive interest varied greatly among the seven common seed predator taxa and were not correlated ($r=0.11$, $P=0.74$, N=7; Figure 2.3, see also S2.3). All mammals except *M. cervinipes* (MMECE) exhibited high levels of both non-destructive and destructive interest in seeds, while the single bird that showed a high level of destructive interest (*A. lathami* (BALLA)) showed a low level of non-destructive interest (Figure 2.3).
Figure 2.3 Behavioural interactions of common predator taxa with seeds. Squares represent mammals, circles represent birds (see table 1 for abbreviations). Axes show the percent of camera days on which a predator was recorded (averaged across 12 sites) where it also showed non-destructive or destructive interest.

2.3.3 Effects of fragmentation on occupancy rates and feeding behaviour

Six of the seven individual common seed predator taxa were significantly affected by habitat fragmentation, however the direction and magnitude of response varied greatly (Figure 2.4a). Among small mammals, *R. fuscipes* (MRAFU) was by far the most commonly recorded taxon in continuous forest but was half as likely to be recorded in fragments, while *M. cervinipes* (MMECE) was only recorded in continuous forest. The large bird *A. lathami* (BALLA) was unaffected by fragmentation whereas the remaining four species (two small birds, one large mammal and one small mammal) all showed significantly higher occupancy rates in fragments than continuous forest (Figure 2.4a).
Figure 2.4 Effect of forest fragmentation on abundance and feeding behaviour of common seed predator taxa (N=6 sites in continuous forest, 6 in fragments; bars show means with SEs). See Table 1 for abbreviations. a) Percent camera days that a taxon was recorded (occupancy rate). b) Percent of these camera days in which there was physical interference with seeds (destructive interest). “N” refers to M. cervinipes not being recorded in fragments. Asterisks show significance levels from t-tests **P<0.001, *P<0.05.

Continuous forest and fragments were clearly separated in the MDS ordination (Figure 2.5), indicating a large difference in the overall taxonomic composition of common seed predators, and this difference was statistically significant (ANOSIM R=0.88, P=0.003). Six taxa were significantly associated with the ordination pattern; two small mammals characterising continuous forest and four taxa from three functional groups characterising fragments (Figure 2.5) in a manner consistent with the species-specific analysis (Figure 2.4a).
Figure 2.5 MDS ordination of among-site variation in seven common seed predator taxa (N=6 sites in continuous forest, 6 in fragments). See table 1 for abbreviations. Input data were the percent camera days on which each taxon was recorded. Lines show statistically significant ($P<0.05$) biplot vectors indicating strength of each taxon’s association with different regions of the plot; $P<0.01$ for all taxa except BCHIN.

The level of destructive behaviour towards seeds that was exhibited by individual taxa also differed between continuous forest and fragments. One species, *R. fuscipes* (MRAFU) showed substantially higher levels of destructive interest in continuous forest than in fragments (Figure 2.4b). Conversely, two species showed much higher levels of destructive interest in fragments: *L. melanoleuca* (BLEME) and *R. rattus* (MRARA) (Figure 2.4b).

Among functional groups containing two or more seed predator species, the only statistically significant effects of fragmentation on abundance were positive: small birds, all birds and all seed predators combined all had higher occupancy rates in fragments; a pattern also seen for small insectivorous birds (Figure 2.6a). Small birds were almost three times as likely to show destructive interest in seeds in fragments than in continuous
forest, whereas small mammals showed less destructive interest in fragments than in continuous forest, while other functional groups showed no significant responses (Figure 2.6b).

**Figure 2.6** Effect of forest fragmentation on abundance and feeding behaviour of common seed predator functional groups and small insectivorous birds (N=6 sites in continuous forest, 6 in fragments; bars show means with SEs). See Table 1 for abbreviations. a) Percent camera days that a functional group was recorded (occupancy rate). b) Percent of these camera days that a functional group was recorded physically interfering with seeds (destructive interest). Asterisks show significance levels from univariate t-tests **P<0.001, *P<0.05.
2.3.4 Levels of physical interference and effects of fragmentation on rates of seed predation

There was a strong positive correlation between the level of behavioural interference recorded by cameras and the percent of deployed seeds which had been physically removed or damaged after five days exposure ($r=0.72$, $P=0.0003$, $N=20$; Figure 2.7). There were trends for higher percentages of large seeds and all seeds to be physically removed or damaged in fragments than in continuous forest (Figure 2.8), but the differences were not statistically significant (P values from t-tests for large, small and all seeds respectively were 0.44, 0.95 and 0.13; N=6 sites in fragments and 6 in continuous forest). The percentage of all seeds (plasticine and real) damaged was highly correated with the percentage of real seeds (not including plasticine) damaged ($r=0.93$, $P<0.0001$, $N=12$).

**Figure 2.7** Relationship between behavioural seed interference events recorded by cameras (% total camera days with interference behaviour) and the physical fate of seeds at stations (% seeds damaged or removed). Points represent individual seed species ($N=20$); each represented by the average values across 12 sites.
Figure 2.8 Effect of fragmentation on the physical fate of seeds at stations (% damaged or removed). N=6 sites in continuous forest, 6 in fragments; bars show means with SEs. Large seeds were ≥ 10mm width, small seeds < 10mm.

2.4 Discussion

2.4.1 Seed predator occurrence and feeding behaviour

With the exception of *R. fuscipes*, which was the only taxon to show a strong attraction to seeds, occupancy rates calculated from cameras with seeds corresponded strongly with those without seeds. Therefore the overall occupancy rates from all cameras can be used as a broad indicator of the relative abundance of all other taxa recorded. Of all common seed predators, *R. fuscipes* was the most abundant taxon, as well as exhibiting the highest level of destructive interest in seeds. In the same study region, including some of the same sites, Fine (1995) and Lott (1997) conducted trap-based studies of small mammals. All small mammals recorded in these studies were also recorded in this study, with the exception of *Rattus norvegicus*, recorded once by Fine (2005), while *Mus musculus* and *Isoodon macrourus* were recorded in very low numbers by Lott (1997). Lott (1997) also conducted feeding trials with the same mammals recorded in this study using seeds of *Castanospermum australe* and concluded that *R. fuscipes* and *Trichosurus*
caninus were its major seed predators. This suggests R. fuscipes is a major seed predator in the Big Scrub region.

More generally at functional group level, both the present study and previous studies show that rodents are major seed predators in rainforests globally. For example, in South America DeMattia et al. (2004) found that small rodents, in particular Oryzomys talamancae (family Muridae), Proechimys semispinosus (family Echimyidae) and Heteromys desmarestianus (family Heteromyidae) were the most abundant and prevalent seed predators across nine tree species. Similarly, Sanchez-Cordero and Martinez-Gallardo (1998) identified Chaetodipus spinatus (family Heteromyidae) and Peromyscus mexicanus (family Cricetidae) as major seed predators of two tree species.

The three common ground-active birds identified in this study all also contributed significantly to seed predation. The large bird A. lathami (family Megapodiidae), and the two small birds C. indica and L. melanoleuca, both pigeons (family Columbidae) are all capable of flight, and spend some of their time in above-ground vegetation, but search on the ground for seeds (Marchant and Higgins, 1993; Higgins and Davies, 1996). All three bird species potentially destroy seeds during ingestion or digestion (Marchant and Higgins, 1993; Higgins and Davies, 1996), and none of the seven common granivorous mammal or bird species identified in this study (from families Muridae, Phalangeridae, Megapodidae and Columbidae) are known to commonly cache or hoard seeds. Although some of the mammals picked up seeds, and may be able to move them short distances, camera footage showed individuals only ever exhibiting destructive interest in seeds at stations, not attempting to remove seeds. Seed hoarding by murid rodents (including Rattus and Melomys) was considered to be negligible in experimental studies of seed removal rates in tropical rainforest of southern Asia (Blackham and Corlett 2015) and northern Australia (Osunkoya 1994), where seed removal was also interpreted as
predation. Birds have rarely been investigated as major seed predators in rainforests, as most previous studies have used trapping (Farwig et al., 2008) or exclosure techniques (Iob and Vieira, 2008) that specifically target mammals. Nevertheless, Christianini and Galetti (2007) found that large ground-dwelling tinamou birds (family Tinamidae) and small pigeons (family Columbidae) contribute significantly to rates of seed predation in south American rainforest.

With the exception of *M. cervinipes*, mammals in the present study showed similar rates of non-destructive and destructive interest in seeds, indicating that they investigated the seeds at close range before attempting to consume them. The mammalian seed predators (three rodents and a possum) are all nocturnal feeders that are likely to use olfactory as well as visual cues when assessing potential food items (Strahan, 1995). In contrast, the three common bird seed predators all showed relatively low non-destructive interest in seeds. All are diurnal (Marchant and Higgins, 1993; Higgins and Davies, 1996), were recorded by cameras in daylight and visually assessed the seeds at a greater distance before approaching them for consumption.

2.4.2 Effects of fragmentation on occupancy rates and feeding behaviour

The species composition of post-dispersal vertebrate seed predators was altered substantially by forest fragmentation, but the species-specific responses to fragmentation varied greatly, and included both positive and negative effects. The total occupancy rate across all seed predator taxa was significantly greater in fragments, but only by about 20% (Figure 6a). Fragmentation mainly caused turnover in the types of species (and functional groups) rather than overall decline or increase. Two small mammals, *R. fuscipes* and *M. cervinipes*, suffered reduced abundance in fragments relative to continuous forest. Although greatly reduced in abundance, *R. fuscipes* was recorded in all
fragments, while *M. cervinipes* was not recorded in any fragments. In contrast, the introduced black rat *R. rattus* was more common in fragments.

For the three small mammal species identified in this study, independent abundance information is available from trap-based studies conducted in the study region: Lott (1997) conducted surveys in 1991-92, Stephen (1999) in 1999 and Fine (2005) in 2004-05. These studies recorded *R. fuscipes* in all continuous sites and *M. cervinipes* in most continuous sites, but *R. fuscipes* was not recorded in the majority of fragments and *M. cervinipes* was not recorded in any fragments. Fine (2005) did not record *R. rattus* in any continuous sites while Lott (1997) and Stephen (1999) did not record *R. rattus* in most continuous sites but all recorded *R. rattus* in most fragments.

This study’s relatively higher recording rate of *R. fuscipes* in fragments may be a result of temporal change if populations of *R. fuscipes* in fragments were lower when the trapping studies took place in 1991-92, 99 and 2004-05. Two kinds of environmental changes have occurred recently. First, fragments in the Big Scrub have been subject to considerable restoration work by landcare groups and landholders in the two decades prior to this study, increasing habitat quality in remnants (Parkes et al., 2012). If populations of *R. fuscipes* were substantially reduced as a result of fragmentation, the improved habitat quality may have resulted in population increases in fragments. Second, progressive decline in the dairy industry since the 1950s has been accompanied by substantial tree regeneration, as well as plantation development for an emerging macadamia nut industry (Neilan et al., 2006; Kanowski et al., 2008; Parkes et al., 2012). The regrowth and plantation forests are both likely to have increased connectivity among remnants, which may have allowed *R. fuscipes* to recolonise fragments where it had previously gone locally extinct.
Among the small mammals identified, trapping studies have reported fragmentation-associated declines in *M. cervinipes*, coupled with increased abundance of *R. rattus*, in other geographically separate regions both to the north (Bentley et al., 2000) and south of the Big Scrub (Dunstan and Fox, 1996; Cox et al., 2003). This consistency with the present study’s findings confirms that the effects of fragmentation reported here were not driven by differing within-region spatial locations of fragments and continuous forest. However, in contrast to results found in this study, Bentley et al. (2000) found similar abundance of *R. fuscipes* in corridors, forests and remnants and plantations while Dunstan and Fox (1996) found that abundance of *R. fuscipes* was also affected by increased levels of human disturbance in fragmented warm-temperate rainforest not connected by any corridors. These contrasting results indicate it is possible that *R. fuscipes* may be more tolerant to fragmentation if there are some habitat connections between remnants.

For both small seed-eating and small insectivorous birds, this study found increases in abundance in fragments. The increase in abundance of small ground-active birds found in this study contrasts with fragmented forest in the neotropics. For example, Stratford and Stouffer (1999) found that nine species of terrestrial insectivorous birds either decreased in abundance or became locally extinct following fragmentation, and Sigel et al. (2010) found that terrestrial insectivorous birds were particularly vulnerable to local extinction or reduced abundance in fragments. However, also in fragmented neotropical rainforest, connectivity through habitat corridors was considered important for ground-active insectivorous birds, allowing increased populations to survive across multiple fragments (Martensen et al., 2008). No active restoration or habitat connectivity work had been reported in the neotropical sites investigated by Stratford and Stouffer (1999) or Sigel et al. (2010), and it is possible that contrasting results between these two studies in the neotropics and this study are due to the recent works to restore habitat
quality and connectivity in the Big Scrub. Nevertheless, in Australian subtropical rainforest, Moran (2004) reported that visual/acoustic observation surveys of various species of fruit-eating birds showed either positive, negative or neutral responses to rainforest fragmentation effects. In Moran’s (2004) study, *C. indica* was recorded in similar numbers in continuous forest and remnants, but the remnants had not been the subject of restoration works.

The specific interactions of some predator taxa with seeds were also altered as a result of fragmentation, and changes in their levels of destructive interest showed a similar pattern to changes in occupancy rates. For example, *L. melanoleuca* showed an increase in both occupancy rate and level of destructive interest in fragments. In contrast, the greatly reduced occurrence of *R. fuscipes* in fragments together with its lowered destructive interest substantially contributed to a net overall effect of lower levels of destructive interest in fragments than in continuous forest. The processes underlying the fragmentation-associated change in species-specific destructive interest in seeds are unclear; one possibility is that greater abundance leads to higher intraspecific competition for food and therefore a more rapid and intense response to the addition of seeds.

### 2.4.3 Implications for forest regeneration

Vertebrate seed predators showed high levels of destructive interest in seeds from a range of tree species, at least across the two years and 1680 camera days of this study. The rates of damage and removal of seeds were also high, about 50% after five days (Figure 2.8). Therefore, any changes in the community of ground-active seed-eating vertebrates could strongly influence patterns of tree regeneration. In central American rainforest, Wright et al. (2007) found that predation on large seeds was reduced where hunters had depleted the numbers of large mammalian seed predators, which in turn
resulted in considerable changes in plant species composition between protected and unprotected sites.

In the present study, fragmentation has greatly altered the species composition and feeding behaviour of the Big Scrub’s vertebrate seed predator assemblage, potentially altering patterns of seed survival and plant regeneration. In the Big Scrub, Fine (2005) investigated rates of seed predation on the common tree species *C. australis* and found that predation rates were strongly related to abundance of *R. fuscipes*. Lott (1997) and Fine (2005) suggested that the reintroduction of native rodents to fragments, in particular *R. fuscipes*, would be a useful management action to potentially restore pre-fragmentation patterns of seed survival. However, the present study’s results suggest that *R. fuscipes* populations in fragments may already have begun to recover. Further monitoring would be needed to confirm whether this is the case.

This study has now also revealed an array of other fragmentation-induced changes across multiple seed predator species. This variation in responses of seed predator taxa to fragmentation may result in reduced predation of some tree species in fragments and increased predation in others, depending on the seed preferences of different predator taxa. The relative contributions of individual predator species to rates of seed predation across a community of tree species will be investigated in Chapter 3.
Chapter Three

Community-level patterns of interaction between seed predators and seeds

Abstract

Vertebrates are major seed predators in rainforests, potentially exerting a substantial influence over a plant species’ potential to regenerate. Community-level interaction networks between multiple species of both seeds and seed predators can thus influence rainforest tree species composition. In 12 rainforest sites of subtropical eastern Australia, automated infra-red cameras at experimental seed stations were used to quantify the patterns of interactions between seven individual ground-active vertebrate taxa and seeds of 20 locally common tree species, from 17 genera and 14 families. Across seed species, the level of interest in seeds recorded by different functional groups (small birds, small mammals, large birds, large mammals) correlated strongly with field damage by these groups to plasticine seeds placed together with the actual seeds of each species. The camera data showed that the interactions between seeds and seed predator species were diffuse and complex, with little evidence of specialised interactions. Seed predators were generalist in diet, consuming seeds from a wide range of tree species, and overlapped considerably in the seed species that commonly received their attention. Likewise, seeds of most tree species were consumed by a range of seed predators. The influence of seed size on levels of destructive interest shown by seed predators was variable among taxa, but no significant difference was found in across all predators. The diffuse nature of community-level interactions between rainforest tree species and the predators of their seeds parallels the diffuse interactions reported for rainforest trees and vertebrate frugivores that disperse their seeds.
3.1 Introduction

Vertebrate seed predators have been reported as major seed predators in rainforests. In rainforest of Borneo, Hautier et al. (2010) reported seed survival of 10 tree species was reduced from over 80% when seeds were protected from vertebrates to below 40% when seeds were exposed to vertebrates after 18 days. Furthermore, in south American rainforest, Iob and Vieira (2008) recorded 54% of seeds from one tree species were removed in 48 hours, concluding that rodents are responsible for the majority of seed predation. Species composition of vertebrate seed predator assemblages in rainforests could therefore play an important role in establishing and maintaining patterns of plant recruitment and plant community structure, and may be influenced by individual predator species preferentially consuming the seeds of some tree species over others.

Previous studies investigating relationships between vertebrate seed predators and rainforest plants have used measurements of vertebrate abundance and experiments that exclude vertebrates of particular sizes from seeds, and have mainly targeted potential seed predation by mammals (Farwig et al., 2008; Iob and Vieira, 2008; Hautier et al., 2010). However, exclosure experiments can only separate predation by different seed predators through indirect evidence coupled with logistically demanding field treatments. Using these methods in neotropical rainforests, small (<750 g) rodents from families including Cricetidae, Heteromyidae and Sciuridae have been identified as major seed predators (Crawley, 1992; Hulme, 1998; Dirzo and Mendoza, 2007; Iob and Vieira, 2008). However, Christianini and Galetti (2007) used bird and mammal surveys together with experimental exclosures to show that birds (tinamous and doves) are also major seed predators in neotropical rainforest, while in a fragment of neotropical rainforest, the abundance of larger-bodied (> 2 kg) seed predators, including common agoutis (Dasyprocta sp.) and peccaries (Pecari tajacu) has been depleted due to hunting, and
doves (family Columbidae) have been identified as major seed predators replacing rodents (Lasso and Ackerman 2004).

Seed size has previously been implicated as an important plant trait influencing predation rates by vertebrates. Among predators, morphological restrictions such as gape width of birds or mammalian handling ability may mean that many species are restrained from consuming large seeds. For example, Dirzo et al. (2007) found that two species of small (< 100 g) rodents, *Heteromys desmarestianus* and *Peromyscus mexicanus* preferred seeds with diameters less than 25mm while larger species preferred seeds with larger diameters in neotropical rainforests.

Community-wide interactions between rainforest seeds and seed predators have not been studied previously, although other important plant-animal interactions, especially those between plants and frugivores, have received significant attention. Early theories on plant-frugivore interactions proposed specialised relationships between plants and frugivores, as a result of species-specific coevolution of traits (McKey, 1975; Howe and Eastbrook, 1977). However, more recent research has highlighted the diffuse nature of plant-frugivore interactions at the community level (Levin et al., 2003; Bascompte and Jordano, 2007; Bluthgen et al., 2007) suggesting that traits of plants and animals evolve as a result of interactions between groups of species, rather than between pairs of individual species (Janzen, 1980).

This study uses automated infra-red camera recorders at experimental seed stations to investigate the patterns of interactions at the community level between multiple species of ground-active mammals and birds and a range of different locally common tree species, in Australian subtropical rainforest. The use of infra-red cameras enables direct observation and quantification of interactions between individual seed predator species.
and seeds. The data are used to assess whether specialised seed-predator interactions are occurring, or seed predators are generalist feeders.

3.2 Methods

3.2.1 Study region and site network

The study took place in the rainforests of the “Big Scrub” region of eastern Australia (28°35'-28°48'S, 153°10'-153°31'E), where vegetation has been characterised as warm subtropical rainforest by Floyd (1990). The temperature ranges from 13˚C (mean annual minimum) to 26˚C (mean annual maximum) and rainfall ranges from 1343 mm to 2327 mm, with a distinct wet season occurring from November to May (Bureau of Meteorology 2005).

Fauna surveys conducted in the Big Scrub have identified nine common species of ground-dwelling mammals; *Antechinus stuartii* (family Dasyuridae), *Melomys cervinipes, Rattus fuscipes*, the introduced *Rattus rattus* (all family Muridae) *Parameles nasuta* (family Peramelidae), *Trichosurus caninus, Trichosurus vulpecula* (both family Phalangeridae), *Thylogale thetis* and *Thylogale. stigmatica* (both family Macropodidae) (Frith 1976). Bird surveys in a different Australian subtropical rainforest region in summer and winter of 2001 have recorded 34 species of frugivorous birds (Moran et al., 2004), while 32 rainforest-dependent bird species have been recorded in the Big Scrub (Frith 1976). Big Scrub rainforest contains a high diversity of plant species; no species or plant family is dominant and the flora is typified by widely distributed species belonging to the families Elaeoacarpaceae, Lauraceae, Meliaceae, Moraceae, Sapindaceae, Rutaceae, Meliaceae and Arecaceae (Floyd, 1990).

Twelve sites distributed across an area of about 300km² were used in this study. All sites are likely to have been selectively logged at some point between the late 1800s
and the mid 1900s, but most are unlikely to have been logged during the 50 years prior to the present study. Each study site covered an area of approximately 100 m by 100 m. Six sites were located in the largest rainforest area in the region’s north; four of these were separated by approximately 500 m. All other sites were separated by between 4 km and 10 km, and six were distributed across smaller remnant patches further south.

### 3.2.2 Field data collection

To investigate vertebrate seed predators and patterns of seed predation, seed stations were established at each site. The seed stations used seeds of 20 tree species known from floristic surveys to occur commonly in the study sites; nine were large-seeded ($\geq$ 10 mm) and 11 small-seeded (< 10 mm). A seed station for a given species was established by tethering five seeds, together with an additional similar-sized “seed” constructed from brown plasticine to a central metal peg, with 50 cm of nylon fishing line. Plasticine seeds were used to provide support for using data from cameras as an indication of levels of seed consumption. Two stations for each species were established in each site, totalling 44 stations per site (18 species were used once and two were used twice). Seed stations were at least 50 m apart from any other stations, and at least 100 m apart from a conspecific station. Each station was left in place for five days, after which time all remaining seeds were collected, and their fate recorded.

To record the vertebrate seed predators and their behaviour, one motion-activated infra-red automated video camera was focused on the seeds at one station for each seed species at each site (i.e. a total of 22 camera sessions with seeds at each site). The duration of each camera session was five days. At each of the 12 sites, the 22 camera traps were recording for 110 days at seed stations, for a total sampling effort of 1320 camera days. Most events that activated the cameras’ motion sensors were movements of vertebrate animals.
Field work took place across two field seasons; in September-December 2013 and June-August 2015. Ten seed species were used in the first season, and twelve seed species in the second season (Table 3.1). The second season included repeat use of two species (E. acuminata and C. glaucescens), to enable a test of between-year variability.

Vertebrate taxa recorded by camera traps were classified as seed predators or otherwise in Chapter 2. Interactions between seed predator taxa and seed species were recorded in two ways: from cameras and from marks on the plasticine “seeds”. First, for each camera footage sequence in which an individual animal was recorded at a seed station, interest in seeds was defined as ‘destructive interest’ if an individual physically interfered with seeds. Second, the plasticine seeds retrieved after five days of exposure were examined for teeth or beak marks, as evidence of physical interference. If marks were found, they were compared to teeth and beak morphology from museum specimens of species within each of four functional groups: small birds, small mammals (classified as weighing 1,000 g or less), large birds and large mammals (classified as weighing over 1,000 g). The species within each group were known from Chapter 3.

3.2.3 Data summary and analysis

Predator species were defined as ‘common’ and individually analysed if they occurred in six or more sites. Analysis was conducted at both individual species and functional group levels. To provide an index of predator abundance at each site, the percentage of total camera days (110/site) during which any given vertebrate taxon was present was calculated. This value was then averaged across all 12 sites.
Table 3.1 Seed species used in study and levels of destructive interest shown by all seed predators. ‘Size class’ refers to whether a seed species is classified as small (S) or large (L). Small seeds are <10mm, large seeds are ≥10mm. ‘Field season’ refers to the season that study species were used: season 1 was from September to December 2013, season 2 was from June to August 2015).

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Species</th>
<th>Family</th>
<th>Seed width (mm)^1</th>
<th>Seed weight (g)^2</th>
<th>Size class</th>
<th>Field season</th>
<th>Destructive interest^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mel_aze</td>
<td><em>Melia azedarach</em></td>
<td>Meliaceae</td>
<td>2.06</td>
<td>0.01</td>
<td>S</td>
<td>2</td>
<td>0.49</td>
</tr>
<tr>
<td>Den_cel</td>
<td><em>Denhamia celastroides</em></td>
<td>Celastraceae</td>
<td>2.63</td>
<td>0.02</td>
<td>S</td>
<td>2</td>
<td>0.34</td>
</tr>
<tr>
<td>Tab_pan</td>
<td><em>Tabernaemontana pandacaqui</em></td>
<td>Apocynaceae</td>
<td>2.81</td>
<td>0.04</td>
<td>S</td>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>Eup_lau</td>
<td><em>Eupomatia laurina</em></td>
<td>Eupomatiaceae</td>
<td>3.10</td>
<td>0.02</td>
<td>S</td>
<td>2</td>
<td>0.33</td>
</tr>
<tr>
<td>Gui_sem</td>
<td><em>Guioa semiglauca</em></td>
<td>Sapindaceae</td>
<td>3.23</td>
<td>0.04</td>
<td>S</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>Mal_phi</td>
<td><em>Mallotus philippensis</em></td>
<td>Euphorbiaceae</td>
<td>3.28</td>
<td>0.02</td>
<td>S</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>Atr_cha</td>
<td><em>Atractocarpus chartaceus</em></td>
<td>Rubiaceae</td>
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<td>0.10</td>
<td>S</td>
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<td>0.27</td>
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<tr>
<td>Ehr_acu</td>
<td><em>Ehretia acuminata</em></td>
<td>Boraginaceae</td>
<td>3.88</td>
<td>0.04</td>
<td>S</td>
<td>1,2</td>
<td>0.19</td>
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<td>Bra_ace</td>
<td><em>Brachychiton acerifolius</em></td>
<td>Malvaceae</td>
<td>4.92</td>
<td>0.21</td>
<td>S</td>
<td>2</td>
<td>0.19</td>
</tr>
<tr>
<td>Cin_cam</td>
<td><em>Cinnamomum camphora</em></td>
<td>Lauraceae</td>
<td>5.86</td>
<td>0.05</td>
<td>S</td>
<td>2</td>
<td>0.32</td>
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<tr>
<td>Neo_dea</td>
<td><em>Neolitsea dealbata</em></td>
<td>Lauraceae</td>
<td>6.86</td>
<td>0.19</td>
<td>S</td>
<td>1</td>
<td>0.12</td>
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<tr>
<td>Cry_mic</td>
<td><em>Cryptocarya microneura</em></td>
<td>Lauraceae</td>
<td>10.05</td>
<td>0.26</td>
<td>L</td>
<td>2</td>
<td>0.35</td>
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<tr>
<td>Wil_hue</td>
<td><em>Wilkia huegeliana</em></td>
<td>Monimiaceae</td>
<td>10.20</td>
<td>0.20</td>
<td>L</td>
<td>1</td>
<td>0.38</td>
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<td>Cry_obo</td>
<td><em>Cryptocarya obovata</em></td>
<td>Lauraceae</td>
<td>11.39</td>
<td>0.58</td>
<td>L</td>
<td>1</td>
<td>0.32</td>
</tr>
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<td>Myrtaceae</td>
<td>11.50</td>
<td>0.76</td>
<td>L</td>
<td>2</td>
<td>0.24</td>
</tr>
<tr>
<td>Cry_gla</td>
<td><em>Cryptocarya glaucescens</em></td>
<td>Lauraceae</td>
<td>12.02</td>
<td>1.02</td>
<td>L</td>
<td>1,2</td>
<td>0.30</td>
</tr>
<tr>
<td>Pod_ela</td>
<td><em>Podocarpus elatus</em></td>
<td>Podocarpaceae</td>
<td>12.73</td>
<td>0.72</td>
<td>L</td>
<td>2</td>
<td>0.45</td>
</tr>
<tr>
<td>Cas_alp</td>
<td><em>Castanospora alphandii</em></td>
<td>Sapindaceae</td>
<td>13.79</td>
<td>1.89</td>
<td>L</td>
<td>1</td>
<td>0.37</td>
</tr>
<tr>
<td>Acm_ing</td>
<td><em>Acmena ingens</em></td>
<td>Myrtaceae</td>
<td>14.50</td>
<td>0.79</td>
<td>L</td>
<td>1</td>
<td>0.39</td>
</tr>
<tr>
<td>Cas_aus</td>
<td><em>Castanospermum australe</em></td>
<td>Fabaceae</td>
<td>32.09</td>
<td>13.93</td>
<td>L</td>
<td>2</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1 Seed width=Average distance measured across the shortest side of seeds, calculated across 10 seeds for each species.
2 Seed weight=Ave dry weight of seeds, following oven-drying for 24 hours at 60°C, calculated across 10 seeds for each species.
3 Destructive interest calculated as follows: the percentage of days in which a seed predator showed destructive interest in seeds of a given seed species was calculated using the total number of camera days on which predators were recorded as the denominator; the values were then averaged across the number of sites that predator was recorded in.
Destructive interest (as a measure of relative predation) by each predator species on each seed species was calculated as follows. For each predator-seed-site combination I calculated the percentages of days in which a given taxon showed destructive interest in seeds at each site, using the total number of camera days on which it was recorded as the denominator. These values were then averaged across the number of sites where that predator was at stations of that seed species (ranging from 0 to 12). Destructive interest in small seeds and large seeds was also calculated separately using a similar procedure (pooling data from the component species seed species in each case: nine large seeded, 11 small seeded).

Profiles of relative destructive interest levels across the 20 seed species were graphed for each common predator species, and similar profiles of relative predator interest levels were constructed for each of the seed species. The multivariate pattern of association between predators and seed species was visualised using two-dimensional nonmetric multidimensional scaling (MDS) ordination of the seven common predator species according to their levels of destructive interest in each of the 20 seed species, with Bray-Curtis between seed dissimilarities, using the ‘Vegan’ package, version 2.3-5, with 4,999 iterations in RStudio version 3.1.2. Intrinsic biplot vectors were added to the ordination to display seed species that were significantly (P<0.05 and P<0.10) associated with the ordination pattern. Extrinsic biplot vectors were likewise added to the ordination to display seed families and seed sizes associated with the ordination pattern.

To test if seed size had an effect on destructive interest across all sites, independent samples t-tests (small vs large, N=12 sites for each) were conducted on individual predator species and functional groups.

For each seed species, the percentage of plasticine seeds found with bite marks from each of the four predator functional groups was calculated from the 24 plasticine
seeds deployed across all sites. To test if this percentage was correlated with the camera based measurement of destructive interest, Pearson’s r correlation (N=20) was used for each predator functional group.

3.3 Results

3.3.1 Interspecific patterns of seed predation

Seven predator species were recorded in at least six sites, with occupancy rates highly variable between species. Three small-bodied, ground-dwelling mammalian rodent species were recorded: *Rattus fuscipes* (24.8% of camera days), *Rattus rattus* (11.5%) and *Melomys cervinipes* (1.6%) as well as one large-bodied mammal; the marsupial possum *Trichosurus* sp. (19.7%). Three ground-dwelling bird species were also recorded: one large-bodied species, *Alectura lathami* (occupancy rate, 12.3% of camera days) and two small-bodied species; *Chalcophaps indica* (7.7%) and *Leucosarcia melanoleuca* (6.8%).

High levels of destructive interest were shown by all seed predators, with an average destructive interest (DI) score of 0.51 across all seed species (Table 3.1) However relative rates of destructive interest varied among seed species (Table 3.1): *Mallotus philippensis* (Mal_phi) experienced the lowest levels of destructive interest (DI=0.28) while *Podocarpus elatus* (Pod_ela) experienced the highest levels (DI=0.60).

Most predator taxa showed destructive interest in a wide range of seed species. *R. fuscipes* was the only predator to consume the seeds of all 20 species (Figure 3.1d), while *A. lathami* consumed the seeds of 19 species (Figure 3.1c). *R. rattus* and *Trichosurus* sp. also had very broad diets, respectively consuming the seeds of 17 and 18 seed species (Figure S3.1.1). *L. melanoleuca* consumed the seeds of 10 species (Figure 3.1a), and *C. indica* 11 species (Figure S3.1.1), while *M. cervinipes* had the narrowest diet, consuming
four species (Figure 3.1b). The apparently narrow diet of less common species such as *M. cervinipes* was in part due to its absence from many seed stations, whereas *L. melanoleuca* and *C. indica* did not consume several seed species, even when it was present with them (Figures 3.1 and S3.1). At functional group level, all groups consumed seeds of a wide range of seed species (Figure S3.1.2).

**Figure 3.1** Seed predator profiles (see Table 1 for abbreviations). Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Dashed lines represent average destructive interest calculated across all 20 seed species.

Likewise, most seed species were eaten by a wide range of predator taxa (Figure S3.2.1). However, no seed species was eaten by all seven predators. For example, the seeds of *Melia azedarach* (Figure 3.2a), *Cinnamomum camphora* (Figure 3.2c) and *Acmena ingens* (Figure 3.2e) were all eaten by six of seven predators. The seeds of three species were eaten by just three predator taxa. Of these, *Castanospermum australe* was
heavily predated by only \textit{R. fuscipes} (Figure 3.2f) and \textit{Eupomatia laurina} was heavily predated by only \textit{A. lathami} (Fig. 3.2d).

\textbf{Figure 3.2} Seed species profiles. Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Dashed lines represent average destructive interest calculated across all seven seed predator taxa. Abbreviations for predator taxa are as follows: BCHIN, \textit{C. indica}; BLEME, \textit{L. melanoleuca}; BALLA, \textit{A. lathami}; MMECE, \textit{M. cervinipes}; MRAFU, \textit{R. fuscipes}; MRARA, \textit{R. rattus}; MTRSP, \textit{Trichosurus} sp.
No strong grouping of predator species based on their consumption of seed species was visible in ordination analysis (Figure 3.3). However, there were some quantitative associations between predator taxa and seed species. Eight of the 20 seed species were associated with particular predator taxa (four at $P<0.05$, and four at $P<0.10$) (Figure 3.3). The seeds of *C. australe* (consumed by three taxa) and *A. ingens* (consumed by six taxa) were most strongly associated ($P<0.05$) with the diet of *R. fuscipes* (MRAFU) (Figure 3.3). While a range of predator taxa consumed the seeds of *M. azedarach* and *D. celastroides* (Figure 3.2) they were most strongly associated ($P<0.05$) with the diet of *L. melanoleuca* (BLEME) (Figure 3.3). At the family level, two families containing more than one seed species characterised ($P<0.10$) patterns of predator diets (Figure 3.3). Lauraceae, comprising five seed species, was most strongly associated with the diets of *R. fuscipes* and *A. lathami*. Sapindaceae was least associated with the diet of *M. cervinipes*, which was the only predator taxon not to consume any seeds from this family (Figure 3.3). Large seeds were most strongly associated with the diet of *R. fuscipes* (MRAFU) and *A. lathami* (BALLA) (Figure 3.3).

Of all predators, *R. fuscipes* was both the most abundant, and also exhibited destructive interest across the widest range of seed species (Figures 3.1d and 3.4, see also Table S2.2.2). Overall, relationships between seed predators and seed species were very diffuse (Figure 3.4), resulting in a complex network of interactions between predator and seed species.
Figure 3.3 Variation among seven seed predator taxa in patterns of destructive interest shown in each seed species. (see Table 1 for abbreviations). Input data for each taxon was the destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Solid lines ($P<0.05$), dashed lines ($P<0.10$). a) Statistically significant intrinsic biplot vectors indicating strength of each seed species’ association with seed predator diets. b) Statistically significant extrinsic biplot vectors indicating strength of each seed family’s association with seed predator diets, and association of seed size. Circles around predator taxa represent the percentage of total camera days during which any given vertebrate taxon was present, averaged across all 12 sites. Abbreviations for predator taxa are as follows: BCHIN, *C. indica*; BLEME, *L. melanoleuca*; BALLA, *A. lathami*; MMECE, *M. cervinipes*; MRAFU, *R. fuscipes*; MRARA, *R. rattus*; MTRSP, *Trichosurus* sp.
Figure 3.4 Interaction network, graphically representing interactions between seed predator taxa and seed species. Lines between predators and seeds represent levels of destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Lines around predators names represent the percent camera days that a taxon was recorded, averaged across 12 sites. Letters in parentheses indicate functional groups: SB, small birds; LB, large birds; SM, small mammals; LM, large mammals.
3.3.2 Influence of seed size on levels of destructive interest

The effect of seed size on levels of destructive interest varied among predator taxa and functional groups (Figure 3.5). Among mammals, *M. cervinipes* showed ten times more destructive interest in small seeds than in large seeds, while *R. fuscipes* and *Trichosurus* sp. showed an approximately 40% greater destructive interest in large than small seeds. The two small bird species tended to prefer small seeds over large seeds, though this difference was not significant (Figure 3.5). At functional group level, small

![Figure 3.5](image)

**Figure 3.5** Seed size effect on destructive interest shown by seed predator taxa and functional groups (bars show means and standard errors across 12 sites). Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Asterisks represent significance $P<0.05$, crosses represent significance $P<0.10$. Abbreviations for predator taxa are as follows: BCHIN, *C. indica*; BLEME, *L. melanoleuca*; BALLA, *A. lathami*; MMECE, *M. cervinipes*; MRAFU, *R. fuscipes*; MRARA, *R. rattus*; MTRSP, *Trichosurus* sp. Functional groups: SB, small birds; LB, large birds; SM, small mammals; LM, large mammals.

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mammals showed a 22% greater destructive interest in large than small seeds, while a similar response was shown across all mammals (Figure 3.5). This variation among predator species in their responses to seed size resulted in very little difference in overall destructive interest in small and large seeds across all predator species (Fig. 5), and no significant effect of seed weight was found.

3.3.3 Predator behaviour vs physical seed damage

Across the 20 seed species, the percentage of plasticine seeds with bite marks after five days of field exposure was positively associated (r values 0.60-0.85, N=20) with camera based rates of destructive interest shown in seeds, for each of the predator functional groups (Figure 3.6). There is some tendency for non-linearity: at low and high levels of destructive interest, the increase in the percentage of plasticine seeds with bite marks was relatively lower than the increase in destructive interest. Nevertheless, camera-based data on destructive interest can arguably be taken as a useful measurement of relative differences in seed consumption.
Figure 3.6 Strength of association between percent of damaged plasticine seeds and levels of camera based destructive interest for each functional group. Points represent the 20 seed species. Percent of damaged plasticine seeds calculated from the total number of plasticine seeds established across all 12 study sites. Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites.

3.4 Discussion

Across the two years and 1320 camera days of this study, all nine of the Big Scrub’s common ground-dwelling mammals as identified by Frith (1976) were recorded. The results presented here show that four of these mammal species are common seed predators in the region. The three rodent species identified as common seed predators in this study have also been recorded in small mammal surveys in subtropical rainforest further north (Bentley et al., 2000), while in warm temperate Australian rainforest, Dunstan and Fox (1996) recorded R. fuscipes and R. rattus and Cox et al. (2003) has recorded all four mammalian predator species. The three ground-dwelling birds here identified as common seed predators have all been recorded in bird surveys of Australian
The results of this study show that ground-active vertebrate seed predators in Australian subtropical rainforests are generalists with respect to the species of seeds they consume. Specialist relationships involving pairwise predator-seed interactions were completely absent, and there was large dietary overlap between different predator taxa. At a global level, this is the first study to have investigated interactions between the seeds of rainforest trees and their vertebrate predators at a community level. However, comparably diffuse interaction networks have also been revealed by studies of rainforest trees and the vertebrate frugivores that disperse their seeds; these studies have generally reported low levels of specialisation, and high levels of dietary overlap among frugivore species (Bluthgen et al., 2007; Schluening et al., 2011).

There was some variation in the strength of interactions between particular seed species and predator taxa. For example, the small seeds of *Eupomatia laurina* were consumed by only three species, among which by far the most significant predator was the bird *A. lathami* (Figures 3.2 and 3.4). Likewise, the seeds of *Castanospermum australe* were eaten by only three predator taxa, among which *R. fuscipes* was clearly the major predator (Figures 3.2 and 3.4). Similarly, both Lott (1997) and Fine (2005) concluded that native rodents are the major predators of *C. australe*, and that *R. fuscipes* was the dominant predator of *C. australe* seeds, from a combination of mammal trapping and exclosure experiments in the Big Scrub.

Seeds of *C. australe* are very large (Table 3.1), however it seems unlikely that morphological restrictions of predators are the main factor restricting consumption of this seed, since the seed coat is thin and can be easily pierced by rodent incisors. The seeds of *C. australe* contain high concentrations of the toxic alkaloid castanospermine (Saul et al.,
1985; Parish et al., 1998), and the smaller range of predator taxa consuming *C. austral* seeds may be a result of those taxa possessing a higher tolerance for castanospermine.

The high abundance and high levels of destructive interest in the widest range of seed species displayed by *R. fuscipes* suggests that this species contributes more to rates of overall predation experienced by rainforest tree species than any other species of seed predator in Australian subtropical rainforest. Two other mammals, *R. rattus* and *Trichosurus* sp., as well as the large bird *A. lathami* were also relatively abundant and consumed the seeds of a range of seed species, indicating these taxa are also major seed predators. *R. rattus* is an invasive non-native species that was introduced at the time of European colonisation (Long, 2003) and has been widely reported as a major seed predator in other regions globally. For example Shiels and Drake (2011) found that *R. rattus* consumed the seeds of all 12 species from 10 plant families that it was offered in feeding trials in Hawaii. Similarly, Salvande et al. (2006) found that predation by *R. rattus* was as high as 70% on seeds of a common tree species (*Ilex canariensis*) on islands of the Mediterranean. Possum species in the genus *Trichosurus* have also been identified as seed predators in the Big Scrub, where Lott (1997) identified *Trichosurus caninus* as a predator of *C. austral*.

While the effect of seed size on levels of destructive interest varied among predator taxa and functional groups, most of this variation could not be explained by testing for differences between small (<10 mm) seeds, which ranged from 0.1-0.21 g and large (≥10 mm) seeds, which ranged from 0.2-13.93 g. Findings from this study indicated rodents (small mammals, < 1000 g) have a preference for large seeds over small seeds. This contrasts with Blate et al. (1998), who used total seed weight as a measurement of seed size to investigate small (<1000 g) rodent predation on seeds ranging from 0.1 g to 11.6 g, and reported that predation decreased as seed size increased in Indonesian
rainforest, finding a positive correlation between seed size and coat thickness and concluding that most large seeds are better protected physically, and may be more difficult for small rodents (*Maxomys* sp.) to consume them.

Morphological restrictions have been previously identified as predictors of dietary patterns in plant-frugivore interaction networks, for example birds with larger gape width or longer beaks have been shown to commonly consume larger fruits (Moran and Catterall, 2010; Dehling et al., 2016). In the present study, the smaller sized bird predators of seeds showed only a weak tendency towards a preference for smaller seeds. Each of the individual small bird species showed a high degree of destructive interest in one large seed species; *C. indica* in *Wilkea huegeliana* and *L. melanoleuca* in *Podocarpus elatus*. Camera footage of *C. indica* and *L. melanoleuca* interfering with these seed species shows that the birds pecked at the seeds, while not appearing to consume the seeds whole. The seeds of *W. huegeliana* have extremely thin seed coats that could be easily penetrated by a beak, suggesting that *C. indica* may be able to consume parts of the seed, although whether this actually occurs remains unclear. On the other hand, seeds of *P. elatus* are surrounded by thick, hard coats. It is possible that *L. melanoleuca* was unable to penetrate *P. elatus* seeds, leaving them without consuming any part of them.

Overall, the results of this study indicate that the observed diffuse community level patterns of seed predation are likely to be the result of a number of factors, including both the defensive traits of seeds and the ability of predators to physically consume and digest seeds.
Chapter Four

Does variation in seeds’ physical and chemical traits influence post-dispersal predation by vertebrates in rainforest?

Abstract

Seeds deposited on the ground surface experience high rates of predation by vertebrates in rainforests. Variation in the level of predation among seeds of different tree species may be influenced by their physical and chemical traits. This study quantified both the field predation rates and the physical and chemical traits of seeds of 20 tree species from 17 genera and 14 families in subtropical rainforest of eastern Australia. Predation rates by ground-active vertebrates were assessed using experimental seed stations. Physical and chemical traits assessed in the laboratory were: seed width, embryo/endosperm dry weight, fracture resistance, coat thickness, relative coat thickness and chemical antifeedant compounds. Seed size, coat thickness, fracture resistance and chemical properties varied greatly among species. Seeds with higher fracture resistance tended to experience lower predation, but overall seed traits were poor predictors of predation rates. 17 of the 20 study species contained antifeedant chemical compounds from four broad classes: alkaloids, terpenes, polyphenols and polyketides. Literature searches found cytotoxic, antimicrobial and neurological biological activity associated with identified antifeedant chemical compounds. Sixteen species contained cytotoxic compounds, one contained a compound with neurological effects and two contained compounds with both cytotoxic and antimicrobial properties. Most (16 of 20) seed species contained a single characteristic class of antifeedant chemical compound, which contrasts with the wider range reported from vegetative parts. These findings indicate that forest tree species have a range of different combinations of seed traits that can provide defence from predation.
4.1 Introduction

When the seeds of rainforest trees are deposited on the forest floor, they often experience high levels of predation by ground-active vertebrates, and often within a short amount of time following dispersal. For example, in neotropical rainforest, Fleury et al. (2014) reported 70% predation by vertebrates on seeds of four tree species after 30 days exposure. Because high seed predation can lower subsequent recruitment of germinating trees, and thereby influence the species composition of rainforest trees, it is important to understand the factors that influence predation rates. Among different tree species, the rates of vertebrate predation on deposited seeds may be influenced by a range of physical and chemical traits that plants have developed as defences against seed predators (Nakagawa and Nakashizuka, 2004). In the coevolutionary arms race between plants and animals, plants have evolved these various forms of defence, while animals in turn have evolved traits to overcome these defences (Karban and Agrawal, 2002; Johnson et al., 2009; Carmona et al., 2011), so that a wide range of possible combinations of plant defence and predator traits may influence vertebrate predation on rainforest seeds.

Trees produce seeds in a wide range of sizes in rainforests, ranging from the minute seeds produced by orchids (Orchidaceae, width, 0.2 mm; weight, $1 \times 10^{-6}$ g) to the giant seeds of *Lodoicea maldivica* (width, 30 cm; weight, 20 kg) (Harper et al., 1970; Leishman et al., 2000). Morphological constraints of some predators may limit their ability to consume large seeds. For example, Vieira et al. (2003) reported that small rodents (26-45 g) mainly consumed small (<10 mm) seeds in south American rainforest while large (>200 g) rodents consumed small and large seeds (1-51 mm). As well as seed size, seed coat hardness may also act as a physical defence, limiting the access of predators to the seed’s embryo and endosperm (Zhang and Zhang, 2008; Lai et al., 2014).
Chemical defence in the form of secondary chemicals present in seeds can also provide protection from predators. Tens of thousands of secondary compounds have been described in plants performing a range of functions (Wink, 2010), including providing defence against natural enemies (Cardinal-Aucoin et al., 2009) and attracting pollinators (González-Teuber and Heil, 2009). Several studies have suggested that secondary compounds have evolved to perform these functions (Wink and Mohamed, 2003; Wink, 2010; Kroymann, 2011), although this remains difficult to determine. The vast majority of research investigating plant secondary compounds has focused on plant defence against herbivores, particularly looking at the compounds found in leaves, where a range of secondary compounds that are toxic to animals have been found, including large numbers of alkaloids, phenolics, terpenes, polyketides and toxic amino acids (Agrawal and Fishbein, 2006; Ness et al., 2009; Spalinger et al., 2010; Mithofer and Boland, 2012; Moore et al., 2014).

The large body of research investigating the physical and chemical defences of rainforest plants has largely focused on leaves rather than seeds. Read et al. (2009) reported that concentrations of phenolic compounds were positively correlated with leaf toughness in 125 plant species across temperate woodlands and tropical rainforests in New Caledonia and Australia, and although high variability was found among species, concluded that plants invested heavily in defence against herbivores. In a wide-ranging study of herbivory defence investigating physical and chemical defence in 261 plants species from 80 families, and across a variety of habitats around the world, Moles et al. (2013) found that plants used a range of different combinations of physical and chemical traits in leaf defence against herbivores.

There has been a lack of research investigating the relative contributions of physical and chemical traits to seed defence. Tiansawat et al. (2014) measured total
quantities of potential chemical defence compounds in seed coats (including phenols, alkaloids and flavonoids) and seed coat thickness in ten species (Macaranga sp.) of Asian rainforest trees, finding high variability among species in both seed coat thickness (1.8-24.6 μm) and the number of compounds found in each species (1-20). Finkelstein and Grubb (2002), in a study of 60 tree species in Australian rainforest found that seeds with greater lipid content were more likely to contain toxins and were better protected physically. Few studies have investigated the relationship between field predation and seed traits, although Fleury et al. (2014) reported that seed mass and coat hardness had no effect on rates of predation by rodents (families Cricetidae and Dasyproctidae) ranging in size from 100-4000 g, on seeds of four tree species in neotropical rainforest. Lai et al. (2014), in a study comparing small (<100 g) rodent (family Muridae) predation on six species of seeds ranging in weight from 0.05 to 0.60 g, found highest predation rates on two species weighing 0.29 g and 0.30 g. However, no study has targeted seeds from a community of tree species, and measured multiple physical and chemical traits.

This study quantifies a range of physical and chemical traits of seeds from 20 species of coexisting rainforest tree species from Australian subtropical rainforest, and also measures the field rates of predation on these seeds by ground-active vertebrates. These data are used to address the question of whether field predation rates on rainforest seeds can be predicted on the basis of their physical and chemical traits.

4.2 Methods

4.2.1 Study region

Field measurements of seed predation rates were investigated in the “Big Scrub” region of eastern Australia (28°37'S, 153°20'E). The climate is subtropical, ranging from 13°C (mean annual minimum) to 26°C (mean annual maximum) and rainfall ranges from 1343 mm to 2327 mm, with a distinct wet season occurring from November to May
Rainforest in the region has been classified as warm subtropical rainforest (Floyd, 1990).

In Chapter 2, seven vertebrate taxa were identified as common post-dispersal seed predators in rainforests of the Big Scrub: three small mammal rodents (*Rattus fuscipes*, *Rattus rattus* and *Melomys cervinipes*), one large marsupial mammal (the possum *Trichosurus* sp.), one large bird (*Alectura lathami*) and two small birds (*Chalcophaps indica* and *Leucosarcia melanoleuca*). Further details of study region and site network are provided in Chapters 2 and 3.

### 4.2.2 Field measurement of predation rates

Predation rates on the seeds of 20 rainforest tree species were assessed using experimental seed stations at each of 12 sites in Big Scrub rainforest, spread across a total area of approximately 300 km$^2$. The selected 20 tree species were known to occur in at least six of the 12 study sites, with at least 10 individuals recorded in total during previous floristic surveys (McKenna unpublished). Species were selected based on similar representation of large ($\geq 10$ mm) and small (<10 mm) seeds. All seeds were sourced from native plant nurseries located within the study region or the Sunshine Coast hinterland (approx. 280 km to its north). Seeds were collected throughout 2013-2014, and all were stored in a freezer at -20°C until data collection was carried out. Storing seeds in the freezer was considered sufficient to maintain the chemistry of the seeds in a stable condition (A. Carroll, pers. comm., 2017).

Levels of seed predation were investigated by establishing seed stations within a local area of about 1.0 ha at each site. A seed station for a given species consisted of five seeds, together with an additional “seed” constructed from plasticine, all of which were tethered to a central metal peg, using 50 cm of nylon fishing line. Two seed stations (ten actual seeds and two plasticine seeds) for each species were established in each site,
totalling 44 stations per site (18 species were used once and two were used twice). Seed stations were at least 50m from any other stations, and 100m from a conspecific station. After establishment, each station was left in place for five days, after which the fate of each seed was recorded, as: “intact” (if no detectable evidence of any physical interference), “damaged” (when there was evidence of physical interference, such as teeth marks on the seed coat or partial removal of endosperm or embryo), or “removed” (if completely absent).

Field work occurred across two field seasons; in September-December 2013 and June-August 2015. Ten seed species (Acmena ingens, Castanospora alphandii, Cryptocarya glaucescens, Cryptocarya obovata, Ehretia acuminata, Guioa semiglauc a, Mallotus philippensis, Neolitsea dealbata Tabernaemontana pandacaqui and Wilkkea huegeliana) were used in the first season, and twelve seed species (Acmena hemilampra, Atractocarpus chartaceus, Brachychiton acerifolius, Castanospermum australe, Cinnamomum camphora, Cryptocarya glaucescens, Cryptocarya microneura, Denhamia celastroides, Ehretia acuminata, Eupomatia laurina, Melia azedarach and Podocarpus elatus) in the second season. The second season included repeat use of two species (E. acuminata and C. glaucescens), to enable a test of between-year variability.

4.2.3 Measurement of physical seed traits

Physical traits were measured using 10 seeds of each species. Any fleshy tissue surrounding seeds was removed. Seed width was recorded as the shortest axis of each seed. Seed coat thickness was measured by first splitting the seed in half and measuring the thickness of the visible seed coat (all the tissue surrounding the embryo and endosperm) with callipers. Fracture resistance was measured using a Geotester dial penetrometer with manual application of force until the point of seed coat fracture was observed, and the weight required was then recorded. Fresh seeds were separated into two
components, embryo/endosperm (E/E) and seed coat. Seed components were then oven-dried at 60°C for 24 hours, and the dry weights were recorded.

4.2.4 Measurement of chemical seed traits

For the following species, seed coats were considered unlikely to be consumed by seed predators and removed prior to chemical analysis: Podocarpus elatus, Cryptocarya glaucescens, Cryptocarya microneura, Cryptocarya obovata, Acmena ingens, Acmena hemilampra, and Castanospora alphandii. For all other species, analysis of chemical traits was conducted on seeds and seed coats. Oven-dried seeds were ground to a fine powder using a mortar and pestle, and for each species, 10 g of ground material was collected for sampling. Samples were then placed in 50 ml of methanol (MeOH), and sonicated for 15 minutes with the resulting supernatant transferred into a separate flask. This step was repeated twice more with MeOH, then twice more with dichloromethane (DCM) before the supernatants were combined and evaporated.

Following evaporation, 1 g of extract from each species was adsorbed onto C18 silica gel (1g, Davasil C-18 bonded silica gel 35-70 μm) by firstly redissolving the extract in MeOH (~10 mL), adding the C18 powder and then evaporating the solvent to produce a gel. The extract adsorbed gel was placed in a HPLC pre-column cartridge (10 mm x 20 mm), connected in series to a C18–bonded silica HPLC column (Thermo Scientific BetaSil 5μm, 21 mm x 150 mm) and eluted with a gradient from 100% H2O to 100% MeOH over 60 minutes followed by isocratic elution for a further 10 minutes at a flow rate of 9.0 mL/min.

Seventy, one-minute fractions were collected, evaporated and fractions analysed by 1H NMR spectroscopy in deuterated dimethyl sulfoxide (d6-DMSO) to determine the purity of individual fractions. Initial data was acquired on a Bruker 500MHz NMR spectrometer at 25°C using 16 scans per analysis. Fractions found to contain pure
compounds were analysed further using a series of 2D NMR experiments (COSY, edited HSQC, HMBC and occasionally ROESY) on a Bruker 800MHz NMR spectrometer. Analysis of correlations obtained from these experiments was used to elucidate the molecular structures of the purified compounds. The molecular structure determinations were carried out by Prof. Anthony Carroll (Griffith University) and followed a logical sequence that involved: a) initial assignment of $^1$H and $^{13}$C chemical shifts for protonated carbons (including assigning these resonances to either CH, CH$_2$ or CH$_3$ groups) from interpretation of correlations observed in an HSQC spectrum, b) connection of these protonated carbons to generate partial molecular structures based upon correlations observed between protons on adjacent carbons in a COSY spectrum and c) connection of these partial structures through analysis of correlations observed in a HMBC spectrum to generate the gross molecular structure. Correlations observed in the HMBC spectrum allowed for the connection of protonated carbons separated from each other by non-protonated carbons since correlations between protons and carbons that are two or three bonds apart are generally obtained from this experiment. The correlations obtained from the above three experimental components, together with a knowledge of how $^1$H and $^{13}$C NMR chemical shifts relate to organic functional groups provided sufficient evidence to establish molecular structures and approximate concentrations for all purified secondary compounds. Three categories of approximate concentrations were defined: <1 mg/g, 1-10 mg/g and >10 mg/g. In some cases, the 3D structure of a compound (including the relative configuration of stereogenic centres within a molecule) was determined from interpretation of correlations (2D ROESY experiment) observed between protons that were close to each other through space.

To determine if the chemical compounds identified from molecular structures were previously known, the literature was searched using the Scifinder chemical abstracts search engine, with the molecular structure obtained from the above-mentioned NMR
experiments as the input information. Where molecular structure matches were obtained between compounds identified in this study and literature reports, a comparison of the NMR data found in the literature with that obtained in the current study was made, in order to further confirm the identity of the molecular structures obtained in the study. Each identified compound was then classified into a ‘broad’ class (alkaloid, polyketide, polyphenolic, terpene) and a ‘specific’ class (idolizidine, isoquinoline, flavonoid, lignan, tannin, diterpene, monoterpenic, sesquiterpene), based on molecular structure.

To establish if identified compounds possessed “antifeedant” (potentially deterring consumption by vertebrates) biological activity, a further search of the Scifinder search engine was undertaken. For each of the previously-known compounds, the molecular structure was used as the input, and searches were refined to only include biological activity. For a given compound, publications were read to establish whether a study reported adverse biological activity on vertebrates, defined as any activity that inhibited cellular processes or altered the organ function of the test subjects. All studies found were carried out on vertebrate cells in vitro, with the exception of two studies, which targeted vertebrates in vivo. Where the published literature reported that a given compound exhibited adverse biological activity on vertebrates, the compound was considered to be potentially antifeedant and classified into one of the following categories; antimicrobial, cytotoxic or neurological.

Where no literature reports were found for a given compound, biological activity was classified in one of two ways: either expert opinion was provided by a natural products chemist (A. Carroll, pers. comm., 2016) or if a compound belonged to a specific class where biological activity of another compound was known, molecular structures were compared. If structures were sufficiently similar, compounds from the same specific class were considered to possess the same biological activity.
Finally, a given seed species was assigned a chemical antifeedant score of zero either if no antifeedant compounds were found, or its sole chemical could not be identified; scores of 1, 2 or 3 were assigned to species with antifeedant compounds, based on three categories of approximate concentrations: <1 mg/g, 1-10 mg/g and >10 mg/g, respectively.

4.2.5 Data summary and analysis

Physical traits for each tree species were averaged across the measured 10 seeds for each species, and a relative coat thickness index (as an indication of coat thickness relative to E/E dry weight) was calculated:

\[
\text{Relative coat thickness} = \frac{CT}{3\sqrt{E/E}}
\]

Where: CT is coat thickness and E/E is the embryo/endosperm dry weight.

A protection index (as a measure of overall defence) was calculated for each seed species, by first range-standardising all values for fracture resistance, coat thickness and chemical antifeedant score across the 20 seed species, which re-scaled values for each trait so that the species with the largest value became 1.0, and the smallest became 0.

\[
\text{Protection index} = \frac{(FR + CT)/2 + AC}{2}
\]

Where: FR is fracture resistance for a given species, CT is coat thickness and AC is the chemical antifeedant score.

For each measured variable, a coefficient of variation was calculated as a measure of its amount of variability relative to its mean scores. To explore the relationship among all measured variables, Spearman’s rank correlations and graphical analyses were used.
(N=20 seed species, see also Table S4.1.1). The following seed traits were then used in subsequent statistical analyses; width, E/E dry weight (as an indication of nutritional value), fracture resistance, coat thickness, relative coat thickness index, chemical antifeedant score and protection index.

The pattern of variation among the 20 seed species in their defensive and nutritional traits was described using two dimensional non-metric multidimensional scaling (NMDS) ordination, using each species’ range standardised measurements of fracture resistance, coat thickness, and chemical antifeedant score as input variables; with Euclidean distance in RStudio version 3.1.2. using the ‘Vegan’ package, version 2.3-5, and 4,999 iterations. Biplot vectors were then overlayed on the ordinations to display both the input (intrinsic) traits that were significantly (P<0.05) associated with the ordination pattern, and to indicate which extrinsic (non-input) measurements (relative coat thickness index, protection index, nutritional value) were also associated with the ordination pattern.

To calculate rates of seed predation from the field data, a quantitative “interference score” of zero was assigned to seeds that remained “intact” after five days exposure, and scores of 0.5 and 1.0 were assigned to “damaged” and “removed” scores respectively. Across all seeds (both actual and plasticine), a small proportion (0.05) were assigned a score of 0.5. An overall interference score was then calculated by averaging across the 10 actual and two plasticine seeds, for a given tree species at each site (for the two duplicated species, 20 actual and four plasticine seeds were used). The site-specific interference scores for each seed species were then averaged across the 12 sites to give a single numerical score indicating the overall level of vertebrate predation. Across the 12 sites, interference scores were highly correlated with both percent removed (r=0.92) and
percent damaged or removed ($r=0.98, N=12$ sites in all cases, $P<0.01$). This interference score was also overlayed on the ordination as an extrinsic (non-input) measurement.
4.3 Results

The 20 seed species varied greatly in seed size, nutritional value and attributes related to seed defence (Table 4.1). Seed width varied from *M. azedarach* (2.06 mm) to *C. austral* (32.09 mm) while E/E dry weight (as an indication of nutritional value) ranged from *M. azedarach* (0.01 g) to *C. austral* (13.49 g). Coat thickness and relative coat thickness ranged from *W. huegeliana* (0.05 mm and 0.10, respectively) to *P. elatus* (1.65 mm and 2.66, respectively), while fracture resistance ranged from *C. austral* (0.29 kg) to *E. acuminata* (16.41 kg). The protection index, as an indication of overall defence varied relatively less than the individual components of physical defence, since its coefficient of variation was only 0.33 compared with 0.56 to 1.86 for individual defensive attributes (Table 4.1). Interference scores also showed relatively low rates of variability (coefficient of variation=0.20), ranging from *E. acuminata* (0.20) to *M. philippensis* (0.77) (Table 4.1, see also Table S4.3.1).

Chemical analyses yielded 37 different compounds from the 20 seed species. Matches of chemical structures with previous literature reports were obtained from the Scifinder search engine for 33 of these compounds (between one and 40 papers located for particular compounds). Five species contained alkaloids, two contained polyketides, seven contained polyphenolics and five contained terpenes. Polyphenolics were the most diverse class, comprising 15 compounds across three specific classes (Figure S4.1). Terpenes comprised nine compounds, within three specific classes (Figure S4.3). Alkaloids comprised eight compounds within three specific classes (Figure S4.4) and five individual compounds were polyketides (Figure S4.2). Four species each contained a compound not previously reported in the literature. *E. laurina* seeds contained six closely related lignan compounds, one of which was new (Figure 4.1), with a structure very similar to the known lignans; *C. obovata* seeds contained three closely related
polyketides, one of which was new (Figure 4.1) but with a similar structure to the known polyketides; N. dealbata seeds contained a new sesquiterpene (Figure 4.1) and A. chartaceus seeds contained a new polyphenol (Figure 4.1).

All 33 previously-known compounds were considered to possess antifeedant activity. Literature reports on biological activity were obtained for 14 of these (between one and 15 papers returned for particular compounds), containing either the name or the name and molecular structure of the compound. For a further nine, information was provided by a natural products chemist (A. Carroll, pers. comm., 2016), while the remaining 10 were considered sufficiently similar to compounds where biological activity was known. Among all plant species, potentially antifeedant compounds were found in 17 of the 20 species, and belonged to four broad classes; alkaloids, polyphenols, polyketides and terpenes (Table 4.2, S4.2). Only one plant species (Podocarpus elatus) contained more than a single specific class of antifeedant chemical, containing two chemicals, each from a different broad class (Table 4.2). The seeds of nine tree species contained only one individual antifeedant compound, while the remaining eight species contained 2-6 antifeedant compounds (Table 4.2).

All compounds considered to be potentially antifeedant possessed cytotoxic activity, with the exception of an alkaloid, ibogaine, from T. pandacaqui which has a neurological effect, while compounds from two species (M. philippensis and A. ingens) contained both cytotoxic and antimicrobial activity (Figure 4.1, Table 4.2). Cytotoxic, neurological and antimicrobial activity were all considered to have potentially antifeedant effects. C. australe seeds contained relatively high (>10 mg/g) concentrations of the indolizidine alkaloid castanospermine, a well-studied compound known to be highly toxic to mammals (Saul et al., 1985; Stegelmeier et al., 2008; Orwa et al., 2009) (Figure 4.1).
Table 4.1: Seed species used in study. ‘Width’ is the distance measured across the shortest diameter, averaged across 10 seeds for each species. ‘E/E dry weight’ is weight of embryo/endosperm component of seeds after being oven-dried at 60°C for 24 hours, averaged across 10 seeds for each species. ‘Fracture resistance’ is the weight required to fractures seed coat, averaged across 10 seed for each species. ‘Antifeedant compound’ is the total concentration of compounds containing antifeedant biological activity: 1<1mg/1g, 2=1-10mg/g, 3>10mg/g.

<table>
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<tr>
<th>Abbreviation</th>
<th>Species</th>
<th>Family</th>
<th>Width (mm)</th>
<th>E/E dry weight (g)</th>
<th>Fracture resistance (kg)</th>
<th>Coat thickness (mm)</th>
<th>Relative coat thickness index</th>
<th>Antifeedant compound</th>
<th>Protection index</th>
<th>Interference score</th>
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<td>0.25</td>
<td>0.49</td>
</tr>
<tr>
<td>Ehr_acu</td>
<td>Ehretia acuminata</td>
<td>Boraginaceae</td>
<td>3.88</td>
<td>0.03</td>
<td>16.41</td>
<td>0.42</td>
<td>1.38</td>
<td>1</td>
<td>0.47</td>
<td>0.20</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.53</td>
<td>0.89</td>
<td>6.12</td>
<td>0.54</td>
<td>1.00</td>
<td>2.35</td>
<td>0.50</td>
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<tr>
<td>SD</td>
<td>7.01</td>
<td>2.98</td>
<td>4.60</td>
<td>0.47</td>
<td>0.60</td>
<td>0.86</td>
<td>0.23</td>
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<tr>
<td>Coeff. of var.</td>
<td>2.40</td>
<td>3.16</td>
<td>1.86</td>
<td>0.64</td>
<td>0.60</td>
<td>0.56</td>
<td>0.33</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

1 Relative coat thickness index = for each species, average coat thickness divided by cubed root of average embryo/component
2 Protection index = for each species, sum of average fracture resistance and coat thickness, divided by 2 + antifeedant compound concentration, divided by two
3 Interference score = average damage score of the 10 real seeds and two plasticine seeds for each species at each site, averaged across all 12 sites.
Damage scores: seeds removed: 1, seeds damaged: 0.5, seeds intact: 0
+ Indicates new compounds found in seeds, unable to be classified as antifeedant compounds or otherwise.
* Indicates compound found in seeds is unlikely to be antifeedant compound
Of the four compounds not previously known, the structure of the new compounds in *E. laurina* and *C. obovata* were considered sufficiently similar to the known compounds within the seeds to classify them as antifeedant. The structures of the two new compounds in *N. dealbata* and *A. chartaceus* were unrelated to any other known compounds, and could not be classified as antifeedant compounds. Seeds of 10 species contained >10 mg/g of antifeedant compounds, three species contained 1-10 mg/g and four species contained <1 mg/g (Table 4.2).

**Figure 4.1** Examples of molecular structures of chemical compounds isolated from seeds. Broad classes named, also showing the plant species and family names, and the specific classes of compounds. “New” indicates a compound not previously identified.
Table 4.2 Information on chemical compounds isolated from study species (see Table 1 for abbreviations). ‘Broad’ and ‘specific’ class of compounds refer to classifications based on both molecular structure and biological activity of compounds, established from literature searches. ‘No. of compounds’ is the total number of individual antifeedant compounds found in seeds. ‘Biological activity type’ is the type of antifeedant activity from literature searches. ‘Concentration score’ is the relative score based on total approx. concentration of compounds, ‘AF concentration score’ is the relative score based on total approx. concentration of antifeedant compounds in seeds: 1<1mg/1g, 2=1-10mg/g, 3>10mg/g.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Broad class of compound</th>
<th>Specific class of compound</th>
<th>No. of compounds</th>
<th>Biological activity type</th>
<th>Reference</th>
<th>Concentration score</th>
<th>AF concentration score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pod_ela</td>
<td>Podocarpaceae</td>
<td>Alkaloid</td>
<td>Monoterpene indole</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Barrero et al. (2003)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pod_ela</td>
<td>Podocarpaceae</td>
<td>Terpene</td>
<td>Diterpene</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Kuo et al. (2008)</td>
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<td>1</td>
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<tr>
<td>Cin_cam</td>
<td>Lauraceae</td>
<td>Alkaloid</td>
<td>Isoquinoline</td>
<td>4</td>
<td>Cytotoxic</td>
<td>Mohamed et al. (2010)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cry_gla</td>
<td>Lauraceae</td>
<td>Polyphenolic*</td>
<td>Flavonoid/stilbene</td>
<td>4</td>
<td>Cytotoxic</td>
<td>Hu et al. (2013)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cry_mic</td>
<td>Lauraceae</td>
<td>Polyketide</td>
<td>Polyketide</td>
<td>2</td>
<td>Cytotoxic</td>
<td>Salomon et al. (2001)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cry_obo</td>
<td>Lauraceae</td>
<td>Polyketide</td>
<td>Polyketide</td>
<td>3</td>
<td>Cytotoxic</td>
<td>Dumontet et al. (2004)</td>
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<td>3</td>
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<tr>
<td>Neo_dea</td>
<td>Lauraceae</td>
<td>Terpene</td>
<td>Sesquiterpene</td>
<td>1</td>
<td>Unknown</td>
<td>Pers. comm.</td>
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<td>0</td>
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<tr>
<td>Wil_hue</td>
<td>Monimiaceae</td>
<td>Alkaloid</td>
<td>Monoterpene indole</td>
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<td>Cytotoxic</td>
<td>Pers. comm.</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Eup_lau</td>
<td>Eupomatiaceae</td>
<td>Polyphenolic</td>
<td>Lignan</td>
<td>6</td>
<td>Cytotoxic</td>
<td>Pers. comm.</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Den_cel</td>
<td>Celastraceae</td>
<td>Terpene</td>
<td>Sesquiterpene</td>
<td>2</td>
<td>Cytotoxic</td>
<td>Tian et al. (2013)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mal_phi</td>
<td>Euphorbiaceae</td>
<td>Terpene</td>
<td>Monoterpene</td>
<td>1</td>
<td>Cytotoxic/antimicrobial</td>
<td>Pers. comm.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cas_aus</td>
<td>Fabaceae</td>
<td>Alkaloid*</td>
<td>Idolizidine</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Saul et al. (1985)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Acm_hem</td>
<td>Myrtaceae</td>
<td>Polyphenolic</td>
<td>Tannin</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Pers. comm.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acm_ing</td>
<td>Myrtaceae</td>
<td>Polyphenolic</td>
<td>Tannin</td>
<td>1</td>
<td>Cytotoxic/antimicrobial</td>
<td>Niho et al. (2001)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Bra_ace</td>
<td>Malvaceae</td>
<td>None*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
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</tr>
<tr>
<td>Mel_aze</td>
<td>Meliaceae</td>
<td>Terpene</td>
<td>Diterpene</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Carpinella et al. (1999)</td>
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<td>1</td>
</tr>
<tr>
<td>Cas_alp</td>
<td>Sapindaceae</td>
<td>Terpene</td>
<td>Diterpene</td>
<td>3</td>
<td>Cytotoxic</td>
<td>Zheng et al. (2013)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gui_sem</td>
<td>Sapindaceae</td>
<td>Polyphenolic</td>
<td>Flavonoid/stilbene</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Hu et al. (2013)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tab_pan</td>
<td>Apocynaceae</td>
<td>Alkaloid</td>
<td>Monoterpene indole</td>
<td>1</td>
<td>Neurological</td>
<td>Mash et al. (1995)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Atr_cha</td>
<td>Rubiaceae</td>
<td>Polyphenolic</td>
<td>Other</td>
<td>1</td>
<td>Unknown</td>
<td>Pers. comm.</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ehr_acu</td>
<td>Boraginaceae</td>
<td>Polyphenolic</td>
<td>Lignan</td>
<td>1</td>
<td>Cytotoxic</td>
<td>Minh et al. (2015)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Indicates other compounds were found unlikely to be antifeedant: Cry_gla (cinnamic acid), Cas_aus (diglyceride), Bra_ace (fatty acids).
Seed width and nutritional value were highly correlated, as was coat thickness with both relative coat thickness index and fracture resistance (r values 0.70-0.92, Table 4.3). The three defensive variables of seed coat thickness, fracture resistance and chemical antifeedant score were uncorrelated and were hence used as input variables in multivariate ordination. The overall protection index was most strongly correlated with its component measurements of coat thickness and chemical antifeedant score, as well as relative coat thickness (r values 0.68-0.79, Table 4.3), rather than unassociated measurements of fracture resistance, E/E dry weight or width (r values 0.34-0.42, Table 4.3).

Table 4.3 Spearman’s r correlation matrix of seed traits (N=20 species). ‘Width’ is the shortest diameter, ‘E/E dry weight’ is weight of embryo/endosperm. ‘Fracture resistance’ is the weight required to fracture the seed coat, ‘Antifeedant compound’ represents the concentration of compounds containing antifeedant biological activity.

<table>
<thead>
<tr>
<th></th>
<th>Width (mm)</th>
<th>E/E dry weight</th>
<th>Fracture resistance (kg)</th>
<th>Coat thickness (mm)</th>
<th>Relative coat thickness index</th>
<th>Antifeedant compound</th>
<th>Protection index²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width (mm)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E dry weight</td>
<td>0.92**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fracture resistance (kg)</td>
<td>0.32</td>
<td>0.18</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coat thickness (mm)</td>
<td>0.52*</td>
<td>0.49*</td>
<td>0.70**</td>
<td>1.00</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Relative coat thickness index</td>
<td>0.13</td>
<td>0.01</td>
<td>0.57**</td>
<td>0.82**</td>
<td>1.00</td>
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<tr>
<td>Known antifeedant compound³</td>
<td>0.15</td>
<td>0.20</td>
<td>-0.20</td>
<td>0.18</td>
<td>0.31</td>
<td>1.00</td>
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</tr>
<tr>
<td>Protection index²</td>
<td>0.42*</td>
<td>0.41*</td>
<td>0.34</td>
<td>0.68**</td>
<td>0.72**</td>
<td>0.79**</td>
<td>1.00</td>
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<tr>
<td>Interference score³</td>
<td>-0.08</td>
<td>-0.10</td>
<td>-0.45*</td>
<td>-0.11</td>
<td>-0.06</td>
<td>0.02</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

¹Relative coat thickness index is standardised to remove allometric influence of seed size.
²Protection index=Overall score based on fracture resistance, coat thickness and chemical antifeedant score.
³Interference score=Field-based measurement of extent of vertebrate predator interference after five days exposure.
Significance values: *P<0.10, *P<0.05, **P<0.01.

MDS ordination of the seed species based on their defensive traits showed a spread of species across the ordination space, but no strong clustering, indicating a lack of grouping of species according to seed traits (Figure 4.2). A diffuse group of species (including Cryptocarya obovata, Cryptocarya glaucescens, Castanospora alphandii,
Acmena ingens and Podocarpus elatus) were associated with relatively high values of all defensive traits (Figure 4.2). Many of these species also had high nutritional value, as evidenced by high E/E dry weights (Figure 4.2). In general, seed species with greater nutritional value also had thicker seed coats ($r=0.49$, $P=0.03$, $N=20$), and tended to possess greater overall protection ($r=0.41$, $P=0.06$, $N=20$, Table 4.3, Figure 4.3). However, the E/E was only weakly associated with the ordination pattern ($r=0.09$, $P=0.47$, Figure 4.2). The biplot vector for field predation rates (interference score) was not significantly associated with the ordination based on all defensive traits ($r=0.12$, $P=0.32$, Figure 4.2) and some presumably well protected seeds experienced high predation (eg. Podocarpus elatus) whereas many less well protected seeds suffered only moderate predation (eg. Melia azedarach, Neolitsea dealbata) (Figure 4.2). However, species with lower fracture resistance experienced higher levels of seed predation ($r=-0.45$, $P=0.04$, $N=20$, Figure 4.4).
Figure 4.2 MDS ordination of among-species variation in nutritional and defensive seed traits from 20 species. Input variables were seed coat thickness, and chemical antifeedant score (all range-standardised across species). Solid lines are overlayed intrinsic biplot vectors, all P<0.001. Dashed lines overlayed are extrinsic biplot vectors, all P<0.001. a) Study species names, see Table 1 for abbreviations. b) Embryo/endosperm (E/E) dry weight. c) Interference scores for each species.
Figure 4.3  a) Relationship between seed coat thickness and E/E dry weight, across 20 species. 
b) Relationship between protection index and E/E dry weight, across 20 species.

Figure 4.4  Relationship between field-based predation (interference score) and fracture resistance, across 20 species. Dots represent each seed species.
4.4 Discussion

4.4.1 Chemical characteristics of rainforest tree seeds

Findings from this study and available information on the diversity and concentrations of secondary compounds found in vegetative parts of plants suggest that in general, seeds contain fewer secondary compounds with antifeedant properties than vegetative parts. For example, over 30 compounds comprising mostly terpenes, and many containing antifeedant properties, along with particularly high concentrations of the terpenes camphor and cineole have been reported in the leaves of *Cinnamomum camphora* (Wanyang et al., 1989; Frizzo et al., 2000). In contrast, the seeds of *C. camphora* differed in both the number and broad classes of compounds found from those of the leaves, containing just four isoquinoline alkaloids. Seven alkaloids and 23 lignan compounds, many containing antifeedant properties, have been isolated from the leaves and flowers of *Eupomatia laurina* (Carroll and Taylor, 1990; Carroll, pers. comm.), while in this study six lignan compounds were isolated from the seeds. However, unlike *C. camphora*, the same specific classes of compounds found in the seeds of *E. laurina* are also found in the leaves. The highly toxic indolizidine alkaloid castanospermine was the only secondary compound isolated from *Castanospermum australe*, and has only ever been found in the seeds however multiple alkaloids and terpenes have been identified in the leaves of *C. australe* (Ahmed et al., 1992; Ahmed et al., 1994).

Results suggest that there are similarities between the broad classes of secondary compounds found in seeds and those found in vegetative components at the family level; both the Lauraceae and Sapindaceae have been relatively well studied, showing correspondence with the chemistry of the seeds analysed in this study. For example, large numbers of polyketides, alkaloids and terpenes, all of which were found in seeds from the Lauraceae family in this study, are considered common in species within Lauraceae.
throughout the tropics, including the specific classes of compounds, the sesquiterpenes (Gottlieb, 1972) and isoquinoline alkaloids (Collins et al., 1990) that were found in seeds in this study. The broad class of polyphenolic compounds identified in seeds from the family Sapindaceae have also been identified in species from this family, in several genera (including *Alectryon*, *Allophylus*, *Cardiospermum*, *Paullinia*, *Sapindus* and *Ungnadia*) throughout the tropics globally (Seigler, 1977; Selmar et al., 1990; Thomsen and Brimer, 1997).

Mammals have the ability to open hard, thick seed coats and consume the seeds inside before discarding the seed coats (Kinzey and Norconk 1990; Lambert at al., 2006). The species with seed coats that were not chemically analysed in this study (*Podocarpus elatus*, *Cryptocarya glaucescens*, *Cryptocarya microneura*, *Cryptocarya obovata*, *Acmena ingens*, *Acmena hemilampra*, and *Castanospora alphandii*) all possess thick, distinct seed coats. If seed coats from these species are not consumed by seed predators, these plant species may not be investing in chemical defence in coats. Rather, they may be allocating chemical defence to the E/E components, although this study did not make comparisons of chemical content between thick and thin seed coats. In rainforests of Borneo, up to 20 potential antifeedant chemical compounds have been recorded in very thin (1.8-24.6 μm) seed coats of ten *Macaranga* sp. (Tiansawat et al., 2014), although this study did not report any review of the literature to identify compounds as potentially antifeedant or otherwise. While methods used in this study to identify compounds as potentially antifeedant do have limitations (ie: extrapolating from the literature, as opposed to direct observations of biological activity) this study does provide a more accurate indication of the potential biological activity of isolated secondary compounds than has previously been provided.
4.4.2 Patterns of physical and chemical protection of rainforest tree seeds

Although high variability in measured traits was found among species, larger seeds with greater nutritional value tended to have thicker seed coats. Similar to results found in this study, Blate et al. (1998) reported high variability in seed weight (as a measure of seed size) and coat thickness across 40 Indonesian rainforest tree species, with seed weight ranging from 0.1 g to 11.6 g, and coat thickness ranging from 0.10 mm to 4.65 mm, and also found a positive correlation between seed size and coat thickness.

Results suggest that across a community, plants use a range of combinations of traits acting as defence against seed predators. For example, *C. australe* seeds had the greatest nutritional value, were poorly protected physically, but contained relatively high concentrations of the highly toxic castanospermine. In contrast the small seeds of *E. acuminata* are well protected physically, with seeds of this species producing very hard seed coats, but are not strongly protected chemically. Although larger seeds tended to have thicker seed coats, these seeds were not proportionally investing more in seed coat thickness as a form of defence. Results found in this study are consistent with research investigating physical and chemical defenses against herbivory in leaves, in which 261 plant species from various different habitats worldwide, were reported that plants use various combinations of chemical and physical defences against herbivores (Moles et al., 2013).

4.4.3 Do defensive and nutritional traits predict field predation rates?

No strong predictors of seed predation were found from seed trait measurements, however there was a moderate trend for seeds with higher fracture resistance of seed coats to experience lower levels of predation. Consistent with results found in this study, previous research investigating the influence of seed traits on predation rates has produced variable results. For example, in a study comparing small (100-200 g) rodent
predation on six rainforest tree species ranging in weight from 0.05-1.30 g, Lai et al. (2014) reported that seeds with intermediate weights of 0.29-0.30 g were more likely to be consumed in Chinese rainforest, observing that these seeds also had thinner coats which may have made it easier for the rodents to consume them. Fleury et al. (2014) reported no effect of seed coat hardness on rates of predation by a medium (2000-4000 g) sized rodent (*Dasyprocta leporina*) on four seed species in the neotropics. In south American rainforest, Vieira et al. (2003) did find some evidence of an effect of seed size on predation rates, finding smaller-bodied rodents (26-84 g) preferred small seeds (<10 mm) while medium to large rodents (72-253 g) consumed seeds of all sizes (1-51 mm).

Higher nutritional value did not lead to greater predation in this study. In contrast, Finkelstein and Grubb (2002) determined lipid concentration in seeds of 60 tropical Australian tree species, and concluded that lipid concentration was higher in species that experienced greater predation by the rodent *Uromys caudimaculatus*. Xiao et al. (2006) investigated the effects of nutritional value and tannin content on seeds of six tree species in Chinese rainforest, and found that seeds with greater fat content and lower tannin content experienced higher predation by rodents (family Muridae), while species with similar fat content but higher tannin concentrations were less preferred. Results in this study suggest seeds with high nutritional value are investing more in overall protection, which may explain why higher nutritional value was not associated with higher predation rates. However, in this study embryo/endosperm dry weight was used as a measurement of nutritional value, but may not be a particularly strong measurement of nutrition. Measurements such as fat concentration, as used by Finkelstein and Grubb (2002) and Xiao et al. (2006) may have provided a greater indication of how important nutrition content is in predicting rates of predation.
The majority of vertebrate seed predators in the study region consume the seeds of most species used in this study (Chapter 3). It is likely that different seed predator species have different seed handling abilities and differences in tolerance to various types of secondary compounds. For example, in neotropical rainforests peccaries are unable to digest some seed species with hard coats, but are able to digest other seed species containing a toxic amino acid, while agoutis can consume hard-coated seeds but not the seeds containing the amino acid (Kuprewicz, 2013). However, some predators may consume multiple seed species, possibly as a strategy to mediate the effects of chemical traits in individual seed species. For example, Nersesian and Banks (2012) found that possums consume higher amounts of protein rich foods when their diet contains the toxin cineole, as a method of compensating for the effect of the toxin. The native rodent *Rattus fuscipes* consumed the seeds of all 20 study species, and was identified as the major seed predator of *C. australis*, which was only consumed by three vertebrate seed predators in rainforest of the Big Scrub (Chapter 3). It is possible that *R. fuscipes* mediates the effects of the toxic alkaloid castanopsermine found in *C. australis* by incorporating a large range of seeds in its diet.

Results from this study indicate that no strong predictions of seed predation can be made based on measurements of seed traits. However, seed fate across a community of seed species appears to be dependent on a range of factors, including seed traits acting as defence, seed nutrient content and the capacity of predators to cope with various physical and chemical defences of seeds.
Chapter Five

Impacts of rainforest fragmentation on post-dispersal vertebrate seed predation

Abstract

High rates of post-dispersal seed predation by ground-active vertebrates in rainforests potentially have significant impacts on patterns of plant recruitment. Altered abundances of vertebrates in rainforest fragments may therefore lead to changes in seed predation and altered community-wide patterns of tree recruitment. Previous research investigating the effects rainforest fragmentation on rates of seed predation has largely focused on one or two populations of tree species, with widely varying results. This study used experimental seed stations to investigate how vertebrate predation on seeds of 20 locally coexisting rainforest trees species was affected by rainforest fragmentation in subtropical eastern Australia. Six continuous forest sites and six fragment sites were established across an area of about 300 km², containing seed stations and automated infra-red cameras to assess vertebrate predators. Overall, 46% of seeds were removed from seed stations after five days exposure, and an interference score (calculated as a measurement of seeds removed or damaged) of 0.49 was recorded. High variability was found in interference scores among species, ranging from 0.20 to 0.77, and seed predation was affected by a strong statistical interaction between habitat type (fragment and continuous) and species of seed. Fragmentation effects on seed predation were highly variable among species, varying in both the direction and extent of change. Five individual species experienced significantly greater predation in fragments, and five species significantly less, than in continuous forest, but these differences were unrelated to seed size. Community-wide patterns of plant recruitment are therefore likely to change in rainforest fragments, as a consequence of turnover in predator species.
5.1 Introduction

Fragmentation has been considered one of the foremost threats to biodiversity in rainforest ecosystems throughout the world, and species composition of rainforest vertebrate assemblages can be substantially altered by fragmentation (Haddard et al., 2015; Santo-Silva et al., 2016). Plant species can experience heavy seed predation by vertebrates in rainforests, where rates of predation can be above 50% within 30 days following dispersal (DeMattia et al. 2004). Changes in species assemblages of rainforest vertebrates have been found to alter rates of seed predation in South American rainforest, where the combined effects of poaching and fragmentation have resulted in large (approx. 30 kg) mammalian seed predators suffering severe population declines; this has led to increases in rates of rodent (families Muridae and Dasyproctidae) seed predation, and higher overall rates of predation on a widespread palm species (Euterpe edulis) (Galetti et al., 2015). Changes to rates of seed predation can ultimately lead to changes in patterns of seedling recruitment and potentially long term changes in plant community composition (Klinger and Rejmanek et al., 2013; Effiom et al., 2016). For example, Wright et al. (2007) found increased seedling densities of large-seeded (100 g) plant species in a central American forest that had experienced high levels of hunting, concluding that hunting of large-bodied (>30 kg) seed predators had released large-seeded rainforest tree species from seed predation.

Previous studies investigating the effects of fragmentation on rates of seed predation in a variety of forest types have focused on seeds from a small number of plant species, and produced contrasting results. Daummann et al. (2008) found almost no predation on one tree species (Strychnos madagascariensis) in forest fragments of Madagascar following the decline in abundance of native rodents (family Nesomyidae). Similarly, in fragments of South American rainforest, lower densities of rodent (family
Cricetidae) populations were associated with low rates of predation in one tree species (*Cabralea canjerana*) compared to continuous forest (Pizo, 1997). On the other hand, Garcia and Chacoff (2007) found higher seed predation on one tree species (*Crataegus monogyna*) in temperate forest in Spain, while in South American rainforest, two tree species experienced greater seed predation in fragments relative to continuous forest, associated with increased activity of rodents (family Heteromyidae) (Guariguata et al., 2002). Changes in abundance of seed predator species may not necessarily lead to any changes in rates of predation, for example in tropical Australia, a large-bodied (approx. 1 kg) rodent (*Uromys caudimaculatus*) suffered reduced abundance in fragments but was still able to consume all available seeds, which resulted in no significant differences in seed predation in fragments (Harrington et al., 1997).

Effects of fragmentation on rates of seed predation may be related to seed size. Vertebrates with larger body sizes tend to be at greater risk of reduced abundance or extinction in fragments than small-bodied vertebrates (Cardillo and Bromham, 2001; Gilbert and Setz, 2001; Vetter et al., 2011), which may result in differentially reduced predation on large-seeded species. Dirzo and Mendoza (2007) reported that predation by small (<1000 g) rodents was higher on small (15 mm) seeds than on large (30 mm) seeds in a fragmentated central American rainforest with reduced populations of larger-bodied (>7 kg) mammals, suggesting that large seeds were likely to experience less predation in areas where populations of medium- large–bodied mammals, with a greater potential to consume large seeds, have been reduced. Potential differences in the effects of fragmentation on seeds of different sizes have rarely been investigated, although Donoso et al. (2003) found variable fragmentation effects on two seed species, reporting increased seed predation by rodents on one large-seeded (18 mm) species in fragments while a second small-seeded (6 mm) species was less affected. The effects of rainforest
fragmentation on post-dispersal seed predation across a range of coexisting rainforest tree species of varying size have not previously been studied.

The subtropical rainforests of eastern Australia have been extensively cleared since European settlement, creating small fragments scattered across a highly modified landscape. Previous research in this region has found that ground-active rodents show a range of positive and negative species-specific responses to rainforest fragmentation, associated with substantial differences in species composition of rodent assemblages between fragments and continuous forest (Lott, 1997; Bentley et al., 2000; Fine, 2005).

The present study used experimental seed stations to assess and compare the rates of predation by ground-active vertebrates on the seeds of 20 locally occurring rainforest tree species, between sites in continuous and fragmented rainforest, in subtropical Australia. Automated infra-red cameras were simultaneously used to assess the seed predator activity at these sites. Analyses focus on the following questions; 1) What are the effects of seed species and forest fragmentation on rates of seed predation? 2) Does seed size influence the effects of fragmentation on seed predation? 3) Do these effects on seed predation correspond with altered seed predator assemblages?

5.2 Methods

5.2.1 Study region and site network

This study was conducted in a region of eastern Australia known as the “Big Scrub” (28°35'-28°48'S, 153°10'-153°31'E). Vegetation in this region has been characterised as warm subtropical rainforest by Floyd (1990). Twelve study sites spread throughout the region across an area of approximately 300 km² were selected for the present study: six in continuous forest (75-150 ha) and six in fragments (4-21 ha) (Figure
5.1. Each study site covered an area of approximately 100 m x 100 m (10,000 m²). Further details of the study region and site network are provided in Chapters 2 and 3.

Remnants of the Big Scrub have been classified as belonging to the *Argyroderon trifoliolata* (White Booyong) floristic alliance, which is characterised by widespread canopy tree species that include *Anthocara* *pa nitidula*, *Argyroderon trifoliolatum*, *Dendrocnide excelsa*, *Ficus macrophylla*, *F. watkinsiana*, *Geisssois benthami* and *Sloanea woollsii* together with a high diversity of other tree species (Floyd, 1990). Other common life forms include vines, epiphytes, climbing aroids, palms and strangler figs in a humid microclimate (Kanowski et al. 2003; Catterall et al. 2004).

![Figure 5.1](image_url)  
**Figure 5.1** Site locations and major water courses (solid lines). Triangles represent continuous sites, squares represent fragments. Dashed line indicates boundary of Big Scrub rainforest prior to European settlement (Floyd 1990).
5.2.2 Field data collection

The seeds of 20 rainforest tree species known to occur in at least six of the 12 study sites, with at least 10 individuals recorded in total during the previous floristic surveys (McKenna, unpublished data) were selected for investigation: nine large-seeded (≥ 10mm) and 11 small-seeded (< 10mm.) (Table 1). Species selection aimed for similar representation of large and small seeds, and was also influenced by species’ availability from local seed suppliers.

To investigate levels of seed predation, two seed stations for each of the 20 tree species were established at each site. Each station comprised five seeds of the selected species and an additional similar-sized “seed” constructed from brown plasticine, all tethered to a central metal peg with 50 cm of nylon fishing line. Eighteen species were used once and two were used twice, giving a total of 44 seed stations per site. Seed stations were at least 50 m apart from any other stations, and at least 100 m from a conspecific station. Each station was left in place for five days, after which the fate of seeds was recorded as: “intact” (if no detectable evidence of any physical interference), “damaged” (when there was evidence of physical interference, such as teeth marks on the seed coat or partial removal of endosperm or embryo), or “removed” (if completely absent).

To record the occurrence of vertebrate seed predators, at each site one automated motion-activated infra-red video camera was focused on the seeds at one station for each seed species and six were established at stations without seeds, giving an overall total of 28 five-day camera sessions at each site (22 camera sessions with seeds, 6 without seeds), and 140 camera days per site (1680 camera days total).

Field work took place across two field seasons; in September-December 2013 and June-August 2015. Ten seed species were used in the first season, and twelve seed species
in the second season (Table 5.1). The second season included repeat use of two species (*E. acuminata* and *C. glaucescens*), to enable a test of between-year variability.

5.2.3 Data treatment and analysis

To calculate rates of seed predation from the field data, a quantitative “interference score” of zero was assigned to actual and plasticine seeds that remained “intact” after five days exposure, and scores of 0.5 and 1.0 were assigned to “damaged” and “removed” scores respectively. An overall interference score was then calculated by averaging across the 10 actual and 2 plasticine seeds, for a given tree species at each site (for the two duplicated species, 20 actual and 4 plasticine seeds were used). The site-specific interference scores for each seed species were then averaged across the 12 sites to give a single numerical score indicating the overall level of vertebrate predation.

For each species at each site, three different measurements were calculated; the percentage of the 10 actual seeds and two plasticine seeds removed, the percentage interfered with (either removed or damaged) and interference score (calculated as above). Average site-specific values of the three predation measurements were also calculated for all small-seeded species, all large-seeded species and all species. Pearson correlation analysis was used to investigate relationships among the percentage of seeds removed, percentage interfered with and interference scores (N=240 site-species combinations). As these measurements were highly correlated, all subsequent analyses use the interference score as the measurement of predation.

Seven vertebrate taxa were recorded in six or more sites in Chapter 2 and defined as “common” seed predator. To calculate the occurrence of common seed predator taxa, at each site the percentage of total camera days where a common predator taxon was recorded was calculated (see also Chapter 2).
A nested analysis of variance (ANOVA) was used to assess the effect of site type (two levels, continuous or fragments) and species (20) nested within seed size (two categories, small or large) on the interference score, with total N=240 site-species combinations, using RStudio version 3.1.2. To test the effects of fragmentation on individual seed species, univariate t-tests were used (fragments vs continuous, N=6 sites in each).

For each seed species, the relative percent change in interference score between the two habitat types (predation turnover) was calculated, using:

\[
\text{predation turnover} = \frac{\text{IF fragment} - \text{IF continuous}}{\max(\text{IF fragment}, \text{IF continuous})} \times 100
\]

Where: IF fragment and IF continuous are the mean interference scores in fragments and continuous forest respectively.

This gives an index that ranges from -100 (in cases where IF is 0 in fragments and 1.0 in continuous forest) to 100 (in cases where IF is 1.0 in fragments and 0 in continuous forest). The correlation between seed species’ interference scores in continuous forest and predation turnover was then investigated, using tree species as replicates.

The pattern of variation in rates of seed predation on seed species among all 12 sites was visualised using two-dimensional nonmetric multidimensional scaling (MDS) ordination, with Euclidean between-site dissimilarities, using the ‘Vegan’ package, version 2.3-5, with 4,999 iterations also in RStudio version 3.1.2. Input data were the interference scores for each seed species at each site. Intrinsic biplot vectors were added to the ordination plot to display individual taxa that were significantly (P<0.05) associated with the ordination pattern. Extrinsic biplot vectors were also added to the ordination to display seed predator taxa significantly (P<0.05) associated with the ordination, using
camera-based data on site-specific frequencies of occurrence of seven previously-identified common seed predator taxa (four mammals and three birds) as described in Chapter 3 (Table S5.3.1). To test if community patterns of seed predation differed significantly between fragments and continuous forest, analysis of similarities (ANOSIM) was used. ANOSIM analysis was also conducted using the ‘Vegan’ package.
Table 5.1 Study species used. ‘Fleshy fruit’ indicates whether or not a tree species produces fleshy fruit and is dispersed by frugivorous birds. ‘Size class’ refers to whether a seed species is classified as small (S) or large (L). Small seeds are <10mm, large seeds are ≥10mm. ‘Field season’ refers to the season that study species were used: season 1 was from September to December 2013, season 2 was from June to August 2015.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Species</th>
<th>Family</th>
<th>Fleshy fruit?</th>
<th>Seed width (mm)</th>
<th>Size class</th>
<th>Field season</th>
<th>Overall Cont</th>
<th>Frag</th>
<th>Interference score</th>
<th>$t^3$</th>
<th>$P^4$</th>
<th>Proportion removed</th>
<th>Overall Cont</th>
<th>Frag</th>
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1Seed width=Average distance across smallest diameter of seeds, calculated across 10 seeds for each species.

2Interference score=Average damage scores calculated from each combination of seed species/study site (12 seeds for each combination). Overall: calculated across all 240 combinations of seed species/study site; Cont, frag: calculated across 120 combinations of seed species/study site in continuous forest and fragments respectively. Damage scores recorded as 1 (seed removed); 0.5 (seed showing evidence of physical interference); 0 (seeds showing no evidence of physical interference).

3,4t values and P values calculated from t-tests for interference scores of each species (fragment vs continuous forest, N=6 sites in each).

5Proportion removed=Average proportion of seeds removed from seed stations calculated from each combination of seed species/study site (12 seeds for each combination). Overall: calculated across all 240 combinations of species/site; Cont, frag: calculated across 120 combinations of species/site in continuous forest and fragments respectively.
5.3 Results

5.3.1 Overall predation rates

High rates of seed predation were recorded following five nights exposure across all 240 combinations of seed species and sites. On average, 46% (range 17-76%, N=12 sites, see also Table S5.1) of seeds were completely removed, 51% (range 23-78%, see also Tables S2.4.1) were interfered with and the interference score was 0.49 (range 0.20-0.77, see also Table S4.3.1). These measurements were highly correlated: r=0.92 for percentage of seeds removed and percentage interfered with; r=0.98 for percentage removed and the interference score and r=0.98 for percentage interfered with and interference score (N=12 sites in all cases, P<0.01).

A nested ANOVA incorporating the joint effects of site type and sampling year, with sites as subjects, on the interference scores of the duplicate species showed no statistically significant effect for C. glaucescens (site type, P=0.60; year, P=0.63; site type x year P=0.96) or E. acuminata (site type, P=0.28; year, P=0.32; site type x year, P=0.39) (Table S5.1.1).

5.3.2 Effects of tree species, fragmentation and seed size

Rates of predation varied greatly among the 20 species (Table 5.1). Across all sites E. acuminata experienced least predation (interference score 0.20), while M. philippensis experienced highest predation (interference score 0.77). Predation rates were similar for small seeds and large seeds (respective interference scores 0.50, 0.47; ANOVA P=0.18; Tables 5.1 and 5.2) and there was no significant interaction between seed size and site type (P=0.21, Table 5.2). ANOVA also revealed that interference scores were affected by a significant interaction between site type and species (P<0.01, Table 5.2), indicating that the effects of fragmentation on rates of seed predation varied among
species (Table 5.2). This interaction occurred because predation rates were significantly
greater in fragments than continuous forest for five individual species, and significantly
greater in continuous forest than fragments for a further five species (Table 5.1, Figure
5.2). In continuous forest sites, seven species had interference scores that were low
(<0.40), nine had moderate (0.40-0.70) and four had high (>0.70). For both the “low” and
“moderate” categories, further ANOVAs showed no significant interactions, but a
significant effect of both species and site type, with increased predation in fragments
(Table 5.2). For the “high” category, the only significant effect was an opposite effect of
site type (decreased predation in fragments; Table 5.2).

Similarly, across the 20 species there was a strong negative relationship between
their interference scores in continuous forest and the predation turnover between the two
habitats (Figure 5.3); species that experienced high predation in continuous forest had
decreased rates of predation in fragments, while species experiencing low predation in
continuous forest had increased predation in fragments. The greatest reduction in
predation in fragments relative to continuous forest was seen in Castanospermum
australe (-52% turnover and 0.90 interference score in continuous forest) and the greatest
increase was seen in W. huegeliana (+52% and 0.23 interference score in continuous
forest).
Table 5.2 Results of nested ANOVA analyses testing effects of site type (N=2, continuous forest or fragment), site nested within site type (12 sites), seed size (small or large) and 20 species nested within seed size on interference scores (IF). Low: Interference score: <0.40 (7 species); Moderate interference score: 0.40-0.70 (9 species); High interference score: >0.70 (4 species). ‘Site type effect size’ refers interference scores in fragments minus the score in continuous forest.

<table>
<thead>
<tr>
<th>Factor</th>
<th>All species</th>
<th>Low IF in cont. forest</th>
<th>Mod. IF in cont. forest</th>
<th>High IF in cont. forest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df  F    P</td>
<td>df  F    P</td>
<td>df  F    P</td>
<td>df  F    P</td>
</tr>
<tr>
<td>Site type</td>
<td>1  2.99 0.09</td>
<td>1  25.55 &lt;0.01</td>
<td>1  7.82 &lt;0.01</td>
<td>1  2.49 &lt;0.01</td>
</tr>
<tr>
<td>Site (Site type)</td>
<td>10 1.44 0.17</td>
<td>10 1.41 0.20</td>
<td>10 1.33 0.23</td>
<td>10 0.69 0.72</td>
</tr>
<tr>
<td>Seed size</td>
<td>1  1.40 0.18</td>
<td>1  0.06 0.80</td>
<td>1  1.23 0.27</td>
<td>1  0.93 0.34</td>
</tr>
<tr>
<td>Seed size x site type</td>
<td>1  1.61 0.21</td>
<td>1  0.46 0.50</td>
<td>1  0.57 0.45</td>
<td>1  3.02 0.09</td>
</tr>
<tr>
<td>Species (Seed size)</td>
<td>18 9.27 &lt;0.01</td>
<td>5  3.93 &lt;0.01</td>
<td>7  4.44 &lt;0.01</td>
<td>2  1.00 0.38</td>
</tr>
<tr>
<td>Species (Seed size) x site type</td>
<td>18 5.96 &lt;0.01</td>
<td>5  0.32 0.90</td>
<td>7  2.67 0.45</td>
<td>2  0.69 0.51</td>
</tr>
<tr>
<td>Error</td>
<td>190 60</td>
<td>80</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Site type effect size</td>
<td>0.04 0.18</td>
<td>0.09</td>
<td>-0.34</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.2 Effects of fragmentation on rates of seed predation. Circles represent the 20 seed species investigated, bars around each point are SEs (N=6 sites) (see Table 5.1 for abbreviations). Axes show interference scores in either fragments or continuous forest, Asterisks represent significant \( P < 0.05 \) differences in interference scores from t-tests (fragments vs continuous, N=6 in each). Solid line represents equal interference scores in both site types.

Continuous forest and fragments differed significantly in multivariate patterns of seed predation across the 20 seed species (ANOSIM \( R=0.86, P=0.002 \)), and MDS ordination clearly separated sites in continuous forest and fragments (Figure 5.4a). Biplot vectors showed that 12 component species were significantly associated with this pattern, which is consistent with the pattern shown in Figure 5.2, with the same five species significantly affected by fragmentation characterising each of continuous forest and fragments (Table 5.4a). Extrinsic biplot vectors for abundance of seed predator taxa showed two rodents (\textit{Melomys cervinipes} and \textit{Rattus fuscipes}) associated with seed predation patterns in continuous forest sites, while one rodent (\textit{Rattus rattus}), one possum
(Trichosurus sp.) and two small birds (Chalcophas indica and Leucosarcia melanoleuca) were associated with seed predation patterns in fragment sites (Figure 5.4b).

**Figure 5.3** Relationship between interference scores for predation on seeds of 20 tree species in continuous forest and turnover in interference score in fragments relative to continuous forest. Dashed line represents no difference between continuous forest and fragments.
Figure 5.4 MDS ordination of among-site variation (6 sites in continuous forest, 6 in fragments) in rates of seed predation (see Table 1 for abbreviations), according to site-specific predation rate (interference score) on 20 seed species a) Lines show statistically significant ($P<0.05$) intrinsic biplot vectors indicating strength of each species’ association with different sites b) lines show statistically significant ($P<0.05$) extrinsic biplot vectors indicating how abundance of common predator taxa was associated with the variation in site-specific predation patterns (the vector for one bird taxon, *Alectura lathami*, was not significant). The genera of predator taxa are as follows: M, *Melomys* sp; R, *Rattus* sp; T, *Trichosurus* sp; C, *Chalcophaps*; L, *Leucosarcia* sp. “b” are birds, “m” are mammals.
5.4 Discussion

5.4.1 Variation in rates of predation among tree species

Across all sites, and across the two years and 1680 camera days of this study, 51% of seeds were interfered with and 46% were removed from seed stations after five night’s exposure, indicating predation by vertebrate seed predators is likely to be a major cause of seed mortality in subtropical rainforest of Australia. However, the high degree of variability in rates of predation found among species suggest that vertebrate seed predators impose significantly greater limits on plant recruitment of those species that experienced particularly high predation (eg. *Mallotus philippensis*, *Castanospermum australe* and *Cinnamomum camphora*) than those that experienced particularly low predation (e. *Acmena ingens*, *Ehretia acuminata*).

Few studies have investigated rates of seed predation across large numbers of seed species. However, consistent with findings in this study, Blate et al. (1998) recorded highly variable rates of rodent (*Maxomys* sp.) seed predation across 40 species and over 30 days in Indonesian rainforest, finding an average predation rate of 51% among species, ranging from 0 to 100%. Research investigating vertebrate seed predation on one or a few seed species has also produced variable findings. For example, Garcia et al. (2005) targeted seeds of three tree species in temperate forest in Spain, finding rodent (families Cricetidae and Muridae) predation rates ranged from 19% to 70% over four weeks. In south American rainforest, Forget et al. (1999) found 43% rodent (families Cricetidae and Echimyidae) predation on one species after two weeks while Donatti et al. (2009) reported 57% vertebrate predation on a palm species (*Astrocaryum aculeatissimum*) after five days. By targeting seeds of 20 species, findings from this study provide strong evidence that seed mortality among coexisting species resulting from predation by vertebrates in rainforests is highly variable in nature.
5.4.2 Fragmentation effects on rates of seed predation

Clear differences were found in community-wide patterns of seed predation between continuous forest and fragments, because of the species-specific effects of fragmentation in both the direction and extent of difference between these habitats. These species-specific effects may be related to the vertebrate seed predator species turnover observed between continuous sites and fragments (Figure 4b). The abundance of two rodents, *Melomys cervinipes* and *Rattus fuscipes* were both associated with continuous forest. Findings in Chapter 3 indicate *R. fuscipes* is a major seed predator of the five seed species that experienced reduced predation in fragments (*Brachychiton acerifolius*, *Castanospermum australe*, *Cinnamomum camphora*, *Cryptocarya obovata* and *Tabernaemontana pandacaqui*); the reduced abundance of *R. fuscipes* in fragments may have led to a release of these species from heavy predation. Conversely, four predator taxa that increased in abundance in fragments all consumed seeds of the five species that experienced higher predation in fragments (*Acmena hemilampra*, *Castanospora alphandii*, *Mallotus philippensis*, *Melia azedarach* and *Wilkea huegeliana*) (Chapter 3), and increased abundance of these predators may be responsible for the increased predation of these seed species in fragments.

Of the five seed species that experienced significantly lower rates of seed predation in fragments relative to continuous forest, *Castanospermum australe* experienced the greatest reduction. In Chapter 4, *R. fuscipes* was identified as a major predator of *C. australe*. Furthermore, Fine (2005) and Lott (1997) have both previously conducted small-mammal trapping surveys in the Big Scrub, and both investigated fragmentation effects on small mammal abundance and seed predation of *C. australe*. Fine (2005) found that *R. fuscipes* was absent from two of six fragments, and that seed predation rates on *C. australe* were lower in those fragments where *R. fuscipes* was not
Lott (1997) also conducted feeding trials with *M. cervinipes, R. fuscipes, Rattus rattus* and *Trichosurus caninus* using seeds of *C. australe* and concluded that *R. fuscipes* is a major seed predator of seed from this species.

*R. fuscipes* was also a major seed predator of the introduced camphor laurel, *Cinnamomum camphora* (Chapter 3), which also experienced significantly reduced predation in fragments. Stewart (2000) investigated seed predation of *C. camphora* in rainforest regrowth in the same study region and found 100% of seeds were predated overnight, in a manner considered consistent with rodent predation. Outside rainforest remnants of the Big Scrub, woody regrowth is dominated by *C. camphora* (Neilan et al., 2006; Kanowski et al., 2008; Parkes et al., 2012), however *C. camphora* is uncommon within intact rainforests. This study’s findings indicate that predation by *R. fuscipes* is likely to be limiting its recruitment within intact rainforest.

Fragmentation led to substantial changes in rates of seed predation for individual species, but without much difference in overall rates of predation in fragments relative to continuous forest, because of the contrasting species-specific responses. The majority of studies investigating fragmentation effects on rates of predation have been conducted in the Neotropics and have produced ambiguous results. For example, in South American rainforest, Andreazzi et al. (2012) found lower rates of rodent predation on seeds of one palm tree (*Attalea humilis*) in fragments relative to continuous forest. Also in South American rainforest, seeds of two tree species (*Dipteryx panamensis* and *Carapa pianensis*) experienced greater predation in fragments relative to continuous forest, associated with increased activity of rodents (Guariguata et al. 2002). The wide range of differing findings can now be explained by the variable nature of seed predation and the varying effects of fragmentation which are apparent when a large number of tree species are studied simultaneously. Studies of one or a few plant species are insufficient as a basis
for drawing conclusions about the impacts of rainforest fragmentation on post-dispersal seed predation.

As large-bodied seed predators with greater area requirements may be more vulnerable to fragmentation than smaller-bodied seed predators (Cardillo and Bromham, 2001; Gilbert and Setz, 2001; Vetter et al., 2011), it was expected that large (≥10 mm) seeds may be eaten less in fragments than in continuous forest. In Chapter 3, the large (>1000 g) possum Trichosurus sp. showed significantly higher preference for large seeds than small seeds, while the large (>1000 g) bird Alectura lathami showed a moderate preference for large seeds. However, findings from this study showed that the two larger-bodied seed predators either increased in abundance in fragments (Trichosurus sp.) or were unaffected (Alectura lathami), and seed size was not a factor influencing seed predation in continuous forest or in fragments.

5.4.3 Implications for tree regeneration

Changes to rates of seed predation can have implications for plant recruitment, for example Fadini et al. (2009) found fewer seedlings and saplings of a palm species (Euterpe edulis) on a South American rainforest island as a result of increased rodent (family Dasyproctidae) predation rates. In the Big Scrub, Lott (1997) and Fine (2005) found there was an increase in density of C. australi seedlings in fragments, concluding that the release of C. australi from seed predation and seedling herbivory is associated with a shift in floristic composition of young trees. As findings from this study have shown that a suite of plant species will experience increased predation in fragments and a further suite will experience decreased predation in fragments, it is now possible to predict likely changes in plant recruitment in fragments of the Big Scrub and how the long term floristic composition of fragments may change.
Chapter Six

General discussion

6.1 Summary of findings of this thesis

This thesis has investigated community-level ecological interactions between seed predators and seeds of 20 locally common tree species in the Big Scrub subtropical rainforest region of eastern Australia, and the consequences of habitat fragmentation for these interactions. Field investigations using automated cameras and experimental seed stations were used to obtain quantitative data on the species composition and abundance of ground-active vertebrate seed predators, and their patterns of interaction with the various seed species. Laboratory analyses identified the physical and chemical traits of each seed species. Data analyses tested the effects of fragmentation on predator species composition and rates of seed predation.

In Chapter 2, a seed predator community of four ground-active birds and five mammal taxa was identified (mainly at species level). Seven of these taxa were identified as common seed predators, comprising three small rodents (*Melomys cervinipes*, *Rattus fuscipes*, *Rattus rattus*), one large possum (*Trichosurus* sp.) and three birds (the large brush-turkey *Alectura lathami* and the small pigeons *Chalcophaps indica* and *Leucosarcia melanoleuca*). Fragmentation substantially altered the species composition of these vertebrates: *M. cervinipes* was only recorded in continuous forest, and *R. fuscipes* was significantly reduced in abundance in fragments, while *C. indica*, *L. melanoleuca*, *R. rattus* and *Trichosurus* sp. were all significantly more abundant in fragments. Fragmentation also altered the levels of destructive interest shown by individual predator taxa; individual predator taxa tended to show greater destructive interest in the habitat in which they were significantly more abundant.
Chapter 3 described a diffuse and complex network of interactions between individual seed predator taxa and 20 rainforest tree species. All seed predators were generalist in diet, and consumed seeds from a wide range of tree species, exhibiting considerable dietary overlap. Likewise, seeds of most tree species were consumed by a range of seed predators. *R. fuscipes* was the most abundant predator taxon, exhibited highest levels of destructive interest in seeds and consumed seeds of the most (all 20 study species) tree species, indicating that it is the dominant seed predator in intact rainforest of the study region.

Chapter 4 showed that seed size, nutritional value, seed coat thickness, fracture resistance and chemical properties of seeds all varied greatly among the 20 rainforest tree species. No strong predictors of seed predation were found, although seeds with greater fracture resistance tended to experience lower rates of predation. However the coefficient of variation (CV) of seed predation rates was lower than the CV values for any seed trait, indicating that the various tree species use a range of different combinations of seed traits as protection against post-dispersal vertebrate predation.

Chapter 5 showed that overall rates of predation by ground-active vertebrates were high: 46% of seeds were removed from seed stations after five days exposure, but predation was highly variable among species. No significant difference was found in overall rates of seed predation between continuous forest and fragments, however the specific effects of fragmentation on rates of seed predation varied greatly among seed species: five species experienced significantly greater predation in fragments than in continuous forest and five species experienced significantly less. Differences among species in fragmentation effects was unrelated to seed size.
6.2 Effects of rainforest fragmentation on interactions between post-dispersal seed predators and seeds

The three rodents that were identified in this study as common seed predators in the Big Scrub (M. cervinipes, R. fuscipes and R. rattus), have all previously been recorded in trapping studies conducted in the same region (Lott, 1997; Fine, 2005). Lott (1997) also identified R. fuscipes and Trichosurus caninus as seed predators of one species used in this study (Castanospermum australe), consistent with results found here. In the Australian wet tropics, Harrington et al. (1997) identified the larger-bodied rodent Uromys caudimaculatus as a major seed predator of 13 seed species. The prominence of rodent species among the common and important vertebrate seed predators found in this study is also consistent with research in other regions globally, which have frequently considered rodents to be major seed predators in rainforests, mostly in the neotropics (Sanchez-Cordero and Martinez-Gallardo, 1998; Iob and Vieira et al., 2008; Klinger and Rejmanek, 2009; Fleury et al., 2014), although Blate et al (1998) also identified rodents as major seed predators in Indonesian rainforest.

Birds have rarely been targeted as seed predators in previous studies of seed predation, which have used seed feeding trials (Munoz and Bonal, 2008) or exclosure experiments designed to exclude vertebrates of particular sizes to identify potential predators (Farwig, et al. 2008). These methods have tended to focus on mammals as potential seed predators, mostly overlooking any role that birds may play in rates of seed predation. Both the small birds identified as seed predators in this study (C. indica and L. melanoleuca) are from the family columbidae, which is consistent with the small amount of research targeting birds in neotropical rainforest that has identified ground-active doves, also from the family columbidae, as well as tinamou birds (family Tinamidae) as major seed predators (Lasso and Ackerman, 2004; Christianini and Galetti 2007). Results
from this study suggest ground-active birds are major seed predators in rainforests of the Big Scrub. Further research targeting both mammals and birds as potential seed predators could provide useful information about the role birds may be playing in rainforest regions globally.

Although *R. fuscipes* was the most significant seed predator overall, its consumption rates varied greatly between seed species, and on their own would not be sufficient to predict the variation in total predation rates among the 20 seed species. Rather, the observed pattern of variation among seed species is an emergent property of the diffuse and complex network of interactions found between individual predator taxa and seed species. Although no previous research has investigated community-level seed-predator interactions, the diffuse nature of the interaction network found in this study is similar to that reported for plant-frugivore interactions (Levin et al., 2003; Bascompte and Jordano, 2007; Bluthgen et al., 2007). Seed predation rates could not be predicted by measuring physical or chemical defences of seeds or their nutritional value, and no other studies have investigated multiple seed traits across a community of tree species and how they relate to patterns of seed predation.

The two large seed predators identified in this study (the bird *A. lathami* and *Trichosurus* sp.) both increased in abundance in fragments, in contrast to previous research in neotropical rainforests which suggests vertebrates with larger body sizes (> 20 kg) such as peccaries and large cats are most vulnerable to fragmentation. (Chiarello et al. 1999; Gilbert and Setz, 2001; Vetter et al., 2011). The ground-active predator taxa that increased in abundance in fragments in this study may have a greater potential to disperse across the modified habitat surrounding fragments, as has been reported in species of marsupials in the Australian wet tropics (Pahl et al., 1988; Laurance, 1990). Similar to the species-specific effects of fragmentation found in this study, and also in the
Australian wet tropics, Harrington et al. (2001) found a substantially altered species composition of small ground-active mammals in fragments compared to continuous forest, where populations of *R. fuscipes* were also significantly reduced in fragments, however *M. cervinipes* was not significantly affected by fragmentation. In Australian subtropical rainforest, Bentley et al (2000) investigated fragmentation effects on populations of small ground-active mammals, including *M. cervinipes, R. fuscipes* and *R. rattus*, and reported variable responses, finding responses of *M. cervinipes* and *R. rattus* consistent with this study, however *R. fuscipes* was recorded in similar numbers in forest and remnants. Also consistent with results in this study, Dunstan and Fox (1996) found that abundance of *M. cervinipes* and *R. fuscipes* both decreased with small remnant size in forests of the southern subtropics.

Previous studies investigating seed predation in rainforests globally have used seeds from a small number of tree species, finding contrasting results. For example, Dausmann at al. (2008) reported greatly reduced mammal predation on seeds of one tree species (*Strychnos madagascariensis*) in fragments in Madagascar, while Pizo (1997) found low abundance of rodents in fragments and subsequent low predation on one seed species (*Cabralea canjerana*) in fragments compared to continuous forest in Brazil. In contrast, increased rodent seed predation has been reported across two tree species (*Dipteryx panamensis* and *Carapa pianensis*) in fragments in Brazil (Guariguata et al., 2002;). In the Australia wet tropics, Harrington et al. (1997) found no significant differences in seed predation by the rodent (*Uromys caudimaculatus*) on three tree species between fragments and continuous forest. Using the seeds of 20 species simultaneously, the present study also found this full range of species-specific responses. Therefore, the range of contrasting results reported by different studies globally could be a result of the variable nature of seed predation that occurs within any particular community of tree species; if a study focuses on one or a few tree species only, than it may be by random
chance it discovers a particular type of fragmentation effect. Video recording cameras which allow actual seed predation events to be captured (Seufert et al., 2009) are an important emerging tool in this field. Multispecies seed predation research using video camera recorders in different rainforests globally has the potential to clarify whether the within-community variation revealed by the present study is a widespread characteristic of rainforests more generally.

Using this study’s findings for the Big Scrub region, it is now possible to combine the information about altered abundances of vertebrate seed predator taxa between continuous forest and fragments, with habitat-specific data on the interactions between predator taxa and seed species, to reveal how the full interaction networks between trees and predators are affected by fragmentation (Figure 6.1). Fragments experienced a wider spread of interactions between individual predator taxa and seed species than was the case in continuous forest; in fragments, the taxa exhibiting the most influence on predation rates (\textit{R. fuscipes}) is no longer the most common predator taxon, and does not interact so strongly with seed species. However, other predators have both become more common and are interacting with seeds more strongly. This gives what appears to be a higher richness of interactions overall (Figure 6.1).

Seeds of tree species that experienced highest predation in continuous forest experienced the greatest reduction in predation in fragments; specifically \textit{Brachychiton acerifolius}, \textit{Castanospermum australe}, \textit{Cinnamomum camphora}, \textit{Cryptocarya obovata} and \textit{Tabernaemontana pandacaqui}. In continuous forest, these species were heavily predated by \textit{R. fuscipes} (Chapter 3) whereas the reduced abundance of this predator in fragments, combined with its reduced level of destructive interest released these seed species from this heavy predation. In fragments, \textit{R. fuscipes} was not functionally replaced by the seed predators that increased in abundance (\textit{R. rattus}, \textit{Trichosurus} sp.)
Figure 6.1 Fragmentation effects on the seed-seed predator interaction network. Lines between predators and seeds represent levels of destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across six sites in continuous forest, six in fragments). Lines around predators names represent occupancy rates, calculated as the percent camera days that a taxon was recorded, averaged across six sites in continuous forest, six in fragments. Letters in parentheses indicate functional groups: SB, small birds; LB, large birds; SM, small mammals; LM, large mammals.

Destructive interest
- <0.20
- 0.20-0.40
- 0.41-0.60
- >0.60

Occupancy rates
- 1-5
- 6-10
- 11-15
- 16-20
- >20
and the two less common doves; *C. indica* and *L. melanoleuca*). Rather, these predators collectively had a different seed predation profile, which resulted in higher predation rates in fragments of a different suite of plant species, which were not highly predated in continuous forest (*Acmena hemilampra*, *Castanospora alphandii*, *Mallotus philippensis*, *Melia azedarach* and *Wilkea huegeliana*).

6.3 Implications for future rainforests of the Big Scrub

This study’s findings of significantly altered seed predator species composition in rainforest fragments, together with altered patterns of predation on the seeds of a range of species, raise the question of likely future changes in the tree species composition of fragments, and future management actions. For one of this study’s tree species that experienced lower predation in fragments, *C. australae*, Fine (2005) and Lott (1997) reported increased seedling abundance in fragments of the Big Scrub and concluded that reduced seed predation and seedling herbivory of this species was associated with increased recruitment of young trees. This study’s findings indicate that five of 20 studied tree species (including *C. australae*) are likely to experience increased seedling recruitment, while for another five species recruitment would decrease. Over decades, this is then likely to lead to shifts in floristic composition in rainforest fragments. To test such predictions would require ongoing longer term monitoring of densities of seedlings and young trees in both continuous forest and fragments.

Previous trapping studies of ground-active rodent seed predators in the Big Scrub recorded *R. fuscipes* in only two of six fragments in the Big Scrub (Lott, 1997; Fine, 2005), and suggested that *R. fuscipes* could be reintroduced to fragments as a management action with the aim of restoring similar patterns of seed survival to those found in continuous forest. These trapping studies were undertaken in 1991-1992 (Lott, 1997) and 2004-2005 (Fine, 2005), and the findings of the present study found that *R. fuscipes* was
present, even if at low abundance, in all fragments. Substantial restoration work has been conducted by landcare groups and landholders in the Big Scrub in the two decades prior to this study, increasing habitat quality in remnants (Parkes et al., 2012). This increase in quality may be allowing substantially reduced populations of *R. fuscipes* to recover in fragments. In addition, substantial tree regeneration and plantation development for the emerging macadamia nut industry in the region following the decline in the dairy industry since the 1950s has occurred (Neilan et al., 2006; Kanowski et al., 2008; Parkes et al., 2012). The subsequent regrowth and plantation forests are both likely to have increased connectivity among remnants, which may have allowed *R. fuscipes* to recolonise fragments where it had previously gone locally extinct. It is possible that *R. fuscipes* populations are recovering through a combination of recolonization and increased reproduction in remnants. Monitoring of *R. fuscipes* populations in both fragments and continuous forest sites is required to clarify whether its populations are recovering, while further studies focusing on rodent abundances and movement patterns in woody regrowth and plantations in the Big Scrub could also provide useful information about connectivity between remnants.

The absence of negative effects of fragmentation on populations of native bird species also suggests that well-managed rainforest fragments can support a diverse community of ground-active vertebrate seed predators. Management options focusing on protecting and maintaining the habitat quality of existing fragments, by increasing their size, improving habitat quality and increasing connectivity in the surrounding landscape matrix, as proposed by Lott (1997), Stephen (1999) and Fine (2005) may indirectly assist in restoring and maintaining desired patterns of tree recruitment by facilitating recovery of communities of vertebrate seed predator species that resemble those found in continuous forest. This includes both avoiding invasion of forest by the non-native tree *Cinnamomum camphora* by maintaining the range of forest-dependent mammals and
birds that consume its seeds (see Chapters 3 and 5; Stewart, 2000), and avoiding hyper-
abundant recruitment of native tree species such as *C. australis* (by maintaining
populations of *R. fuscipes*).
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Supplementary material

Chapter Two supplementary material

S2.1 Images of three fragments used in study and seed stations

**Figure S2.1.1** Photos of Big Scrub fragments used. a) Dawes Bush b) Davis Scrub c) Booyong Flora Reserve

**Figure S2.1.2** Graphical representation of seed stations established and used to measure field predation rates.
Figure S2.1.3 Seed station established with automated infra-red video camera focused on seeds.
### S2.2 Camera-based data of occurrence of all vertebrate taxa recorded (individual sites)

**Table S2.2.1** Occupancy rates of vertebrate taxa and predator functional groups. Calculated as the percentage of days a given taxon was recorded by cameras with and without seeds (140 camera days/site), averaged across continuous forest and fragments (N= 6 in each) and all sites (N=12). Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Vertebrate taxa</th>
<th>Big Scrub 1</th>
<th>Big Scrub 2</th>
<th>Big Scrub 3</th>
<th>Big Scrub 4</th>
<th>Boomerang Falls</th>
<th>Minyon Falls</th>
<th>Cont av.</th>
<th>Booyong Flora Reserve</th>
<th>Davis Scrub</th>
<th>Dawes Bush</th>
<th>Emery Johnson's Nature Reserve</th>
<th>Victoria Park</th>
<th>Frag av.</th>
<th>Overall av.</th>
</tr>
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<tbody>
<tr>
<td>Accipiter novaehollandiae</td>
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<tr>
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<td>4.3</td>
<td>1.4</td>
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<td>9.3</td>
<td>10.1 (2.2)</td>
</tr>
<tr>
<td>Columba leucomela</td>
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<td>0.0</td>
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<td>2.1</td>
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<td>17.6 (3.2)</td>
</tr>
<tr>
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<td>0.7</td>
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<td>0.1 (0.1)</td>
</tr>
</tbody>
</table>

<sup>1</sup>While <i>Varanus varius</i> and <i>Trichosurus sp.</i> are also native, their occurrence in fragmented landscapes is likely influenced by the introduction of non-native predators. This may affect the occupancy rates observed in these areas.

<sup>2</sup>Thylogale spp. are Marsupial Gliders, which are known to have a high tolerance to fragmentation and are often considered keystone species in fragmented ecosystems.

<sup>3</sup>While <i>Varanus varius</i> is native, its occurrence in fragmented landscapes is likely influenced by the introduction of non-native predators, which may affect the occupancy rates observed in these areas.
<table>
<thead>
<tr>
<th>Seed predator functional groups</th>
<th>Big Scrub 1</th>
<th>Big Scrub 2</th>
<th>Big Scrub 3</th>
<th>Big Scrub 4</th>
<th>Boomerang Falls</th>
<th>Minyon Falls</th>
<th>Cont av.</th>
<th>Booyong Flora Reserve</th>
<th>Davis Scrub</th>
<th>Dawes Bush</th>
<th>Emery's Scrub</th>
<th>Johnson's Nature Reserve</th>
<th>Victoria Park</th>
<th>Frag av.</th>
<th>Overall Av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small birds</td>
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<td>5.3</td>
<td>5.7</td>
<td>7.9</td>
<td>5.0</td>
<td>5.3</td>
<td>6.6 (0.5)</td>
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<td>15.6</td>
<td>25.0</td>
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<td>16.4</td>
<td>18.2</td>
<td>11.5 (2.4)</td>
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<tr>
<td>Large birds</td>
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<td>5.7</td>
<td>10.0</td>
<td>12.1</td>
<td>14.3</td>
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<td>1.4</td>
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<td>33.5 (3.7)</td>
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<td>11.9 (1.8)</td>
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<td>30.0</td>
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<td>56.2 (6.1)</td>
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</tbody>
</table>

1. *M. cervinipes* is the only known *Melomys* species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005)

2. Most likely *T. thetis* however may potentially include *T. stigmatica*

3. Most likely *T. caninus* or *T. vulpecula*
Table S2.2.2 Average percentage of days a given taxon was recorded by cameras with seeds (110 camera days/site), averaged across continuous forest and fragments (N= 6 in each) and all sites (N=12). Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Vertebrate taxa</th>
<th>Continuous forest</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Big Scrub 1</td>
<td>Big Scrub 2</td>
</tr>
<tr>
<td>Accepipter novaehollandiae</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Chalcophaps indica</td>
<td>1.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Columba leucomela</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Eopsaltria australis</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Leucosarcia melanoleuca</td>
<td>2.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Orthonyx temminckii</td>
<td>3.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Pitta versicolor</td>
<td>0.9</td>
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<td>Psophodes olivaceus</td>
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<td>1.8</td>
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<td>Sericornis citreogularis</td>
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<tr>
<td>Sericulus chrysocephalus</td>
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<tr>
<td>Zoothera heinei</td>
<td>0.0</td>
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</tr>
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<td>15.5</td>
<td>6.4</td>
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<tr>
<td>Antechinus sp.</td>
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<tr>
<td>Varanus varius</td>
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</tbody>
</table>

\(^1\)M. cervinipes is the only known Melomys species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005)

\(^2\)Most likely T. thetis however may potentially include T. stigmatica

\(^3\)Most likely T. caninus or T. vulpecula
Table S2.2.3  Average percentage of days a given taxon was recorded by cameras without seeds (30 camera days/site), averaged across continuous forest and fragments (N= 6 in each) and all sites (N=12). Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Vertebrate taxa</th>
<th>Continuous forest</th>
<th>Fragments</th>
<th>Overall</th>
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<tr>
<td></td>
<td>Big Scrub 1</td>
<td>Big Scrub 2</td>
<td>Big Scrub 3</td>
</tr>
<tr>
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<td>Chalcophaps indica</td>
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<td>Columba leucomela</td>
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<td>Eopsaltria australis</td>
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<tr>
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<td>6.7</td>
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<tr>
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<td>0.0</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>3.3</td>
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</tr>
<tr>
<td>Melomys cervinipes(^1)</td>
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</tr>
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</table>

\(^1\)M. cervinipes is the only known Melomys species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005)

\(^2\)Most likely T. thetis however may potentially include T. stigmatica

\(^3\)Most likely T. caninus or T. vulpecula

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### S2.3 Camera-based data of feeding behaviour of all vertebrate taxa recorded (individual sites)

#### Table 2.3.1 Non-destructive interest scores of vertebrate taxa and predator functional groups. Calculated as the percent of camera days a given taxon was recorded where it showed interest in seeds without physically interfering with them, averaged across the number of sites where recorded. ‘N’ represents vertebrate not recorded at a site. Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Vertebrate taxa</th>
<th>Continuous forest</th>
<th></th>
<th>Fragments</th>
<th></th>
<th>Overall av.</th>
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<td>Big Scrub 2</td>
<td>Big Scrub 3</td>
<td>Big Scrub 4</td>
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<td>N</td>
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<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
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<table>
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<th>Fragments</th>
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<td>Large birds</td>
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<tr>
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<td>Large mammals</td>
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1. *M. cervinipes* is the only known *Melomys* species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005).  
2. Most likely *T. thetis* however may potentially include *T. stigmatic*  
3. Most likely *T. caninus* or *T. vulpecula*
Table S2.3.2 Destructive interest scores of vertebrate taxa and predator functional groups. Calculated as the percent of camera days a given taxon was recorded where it physically interfered with seeds, averaged across the number of sites where recorded. ‘N’ represents vertebrate not recorded at a site. Standard errors in parentheses.

<table>
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<tr>
<th>Vertebrate taxa</th>
<th>Big Scrub 1</th>
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<th>Big Scrub 3</th>
<th>Big Scrub 4</th>
<th>Boome-rang Falls</th>
<th>Minyon Falls</th>
<th>Cont av.</th>
<th>Booyong Flora Reserve</th>
<th>Davis Scrub</th>
<th>Dawes Bush</th>
<th>Emery’s Scrub</th>
<th>Johnson’s Nature Reserve</th>
<th>Victoria Park</th>
<th>Frag av.</th>
<th>Overall av.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accipiter novaehollandiae</td>
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<td>N</td>
<td>N</td>
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<td>0.22</td>
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</tr>
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<tr>
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<td>Big Scrub 3</td>
<td>Big Scrub 4</td>
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<td>Minyon Falls</td>
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<td>Dawes Bush</td>
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<td>Overall av.</td>
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<td>0.14</td>
<td>0.62</td>
<td>0.25</td>
<td>0.53</td>
<td>0.37</td>
<td><strong>0.40 (0.07)</strong></td>
<td>0.57</td>
<td>0.50</td>
<td>0.00</td>
<td>0.41</td>
<td>0.42</td>
<td>0.30</td>
<td><strong>0.37 (0.08)</strong></td>
<td><strong>0.38 (0.05)</strong></td>
</tr>
<tr>
<td>Small birds</td>
<td>0.00</td>
<td>0.00</td>
<td>0.17</td>
<td>0.00</td>
<td>0.17</td>
<td>0.33</td>
<td><strong>0.11 (0.05)</strong></td>
<td>0.23</td>
<td>0.32</td>
<td>0.42</td>
<td>0.38</td>
<td>0.25</td>
<td>0.26</td>
<td><strong>0.31 (0.03)</strong></td>
<td><strong>0.21 (0.04)</strong></td>
</tr>
<tr>
<td>Small mammals</td>
<td>0.50</td>
<td>0.35</td>
<td>0.53</td>
<td>0.50</td>
<td>0.43</td>
<td>0.41</td>
<td><strong>0.45 (0.03)</strong></td>
<td>0.40</td>
<td>0.38</td>
<td>0.36</td>
<td>0.41</td>
<td>0.33</td>
<td>0.35</td>
<td><strong>0.37 (0.01)</strong></td>
<td><strong>0.41 (0.02)</strong></td>
</tr>
<tr>
<td>Large mammals</td>
<td>0.27</td>
<td>0.20</td>
<td>0.30</td>
<td>0.10</td>
<td>0.21</td>
<td>0.25</td>
<td><strong>0.22 (0.03)</strong></td>
<td>0.22</td>
<td>0.25</td>
<td>0.41</td>
<td>0.27</td>
<td>0.17</td>
<td>0.43</td>
<td><strong>0.29 (0.04)</strong></td>
<td><strong>0.26 (0.03)</strong></td>
</tr>
<tr>
<td>All birds</td>
<td>0.38</td>
<td>0.08</td>
<td>0.44</td>
<td>0.17</td>
<td>0.43</td>
<td>0.39</td>
<td><strong>0.32 (0.06)</strong></td>
<td>0.31</td>
<td>0.33</td>
<td>0.36</td>
<td>0.43</td>
<td>0.35</td>
<td>0.31</td>
<td><strong>0.35 (0.02)</strong></td>
<td><strong>0.33 (0.03)</strong></td>
</tr>
<tr>
<td>All mammals</td>
<td>0.46</td>
<td>0.35</td>
<td>0.50</td>
<td>0.48</td>
<td>0.43</td>
<td>0.42</td>
<td><strong>0.44 (0.02)</strong></td>
<td>0.36</td>
<td>0.38</td>
<td>0.42</td>
<td>0.41</td>
<td>0.27</td>
<td>0.43</td>
<td><strong>0.38 (0.02)</strong></td>
<td><strong>0.41 (0.02)</strong></td>
</tr>
<tr>
<td>All predators</td>
<td>0.45</td>
<td>0.31</td>
<td>0.51</td>
<td>0.45</td>
<td>0.49</td>
<td>0.46</td>
<td><strong>0.45 (0.03)</strong></td>
<td>0.40</td>
<td>0.39</td>
<td>0.43</td>
<td>0.52</td>
<td>0.38</td>
<td>0.43</td>
<td><strong>0.42 (0.02)</strong></td>
<td><strong>0.44 (0.02)</strong></td>
</tr>
</tbody>
</table>

1. *M. cervinipes* is the only known *Melomys* species known to occur in the rainforests of the study region (Lott and Duggin 1993; Fine 2005)
2. Most likely *T. thetis* however may potentially include *T. stigmatica*
3. Most likely *T. caninus* or *T. vulpecula*
S2.4 Rates of physical seed interference recorded from seed stations (individual sites)

Table S2.4.1 Proportion of seeds removed or interfered with for each seed species. Calculated as the proportion of seeds removed from each combination of seed species/study site (12 seeds for each combination, 24 seeds for duplicate species). Overall: averaged across all 240 combinations of species/site; Cont. frag: averaged across 120 combinations of species/site in continuous forest and fragments respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Big Scrub</th>
<th>Big Scrub</th>
<th>Big Scrub</th>
<th>Big Scrub</th>
<th>Boome-rang Falls</th>
<th>Minyon Flora</th>
<th>Booyong Reserve</th>
<th>Dawes Bush</th>
<th>Davis Emery’s Scrub</th>
<th>Johnson’s Nature Reserve</th>
<th>Victoria Park</th>
<th>Frag av.</th>
<th>Overall av.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Melia azedarach</em></td>
<td>0.67</td>
<td>0.42</td>
<td>0.33</td>
<td>0.50</td>
<td>0.58</td>
<td>0.42</td>
<td>0.49 (0.05)</td>
<td>0.67</td>
<td>0.67</td>
<td>0.75</td>
<td>0.75</td>
<td>0.42</td>
<td>0.67 (0.05)</td>
</tr>
<tr>
<td><em>Denhamia celastroides</em></td>
<td>0.17</td>
<td>0.17</td>
<td>0.58</td>
<td>0.33</td>
<td>0.67</td>
<td>0.33</td>
<td>0.38 (0.06)</td>
<td>0.83</td>
<td>0.58</td>
<td>0.17</td>
<td>0.50</td>
<td>0.33</td>
<td>0.50 (0.09)</td>
</tr>
<tr>
<td><em>Tabernaemontana pandacaqui</em></td>
<td>0.75</td>
<td>1.00</td>
<td>0.67</td>
<td>0.92</td>
<td>0.50</td>
<td>0.83</td>
<td>0.78 (0.08)</td>
<td>0.67</td>
<td>0.33</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42</td>
<td>0.44 (0.05)</td>
</tr>
<tr>
<td><em>Eupomatia</em></td>
<td>0.58</td>
<td>0.58</td>
<td>0.50</td>
<td>0.42</td>
<td>0.17</td>
<td>0.42</td>
<td>0.44 (0.06)</td>
<td>0.33</td>
<td>0.42</td>
<td>0.58</td>
<td>0.42</td>
<td>0.58</td>
<td>0.47 (0.04)</td>
</tr>
<tr>
<td><em>Guioa semiglauca</em></td>
<td>0.08</td>
<td>0.33</td>
<td>0.33</td>
<td>0.50</td>
<td>0.42</td>
<td>0.42</td>
<td>0.35 (0.06)</td>
<td>0.50</td>
<td>1.00</td>
<td>0.08</td>
<td>0.67</td>
<td>0.42</td>
<td>0.51 (0.13)</td>
</tr>
<tr>
<td><em>Mallotus philippensis</em></td>
<td>0.83</td>
<td>0.58</td>
<td>0.67</td>
<td>0.50</td>
<td>0.50</td>
<td>0.60 (0.05)</td>
<td>0.92</td>
<td>0.83</td>
<td>1.00</td>
<td>1.00</td>
<td>0.75</td>
<td>1.00</td>
<td>0.92 (0.04)</td>
</tr>
<tr>
<td><em>Atractocarpus chartaceus</em></td>
<td>0.75</td>
<td>0.58</td>
<td>0.25</td>
<td>0.58</td>
<td>0.67</td>
<td>0.58</td>
<td>0.57 (0.07)</td>
<td>0.58</td>
<td>0.33</td>
<td>0.25</td>
<td>0.42</td>
<td>0.67</td>
<td>0.44 (0.06)</td>
</tr>
<tr>
<td><em>Ehretia acuminata</em></td>
<td>0.08</td>
<td>0.17</td>
<td>0.33</td>
<td>0.29</td>
<td>0.13</td>
<td>0.17</td>
<td>0.19 (0.03)</td>
<td>0.38</td>
<td>0.13</td>
<td>0.17</td>
<td>0.38</td>
<td>0.42</td>
<td>0.27 (0.05)</td>
</tr>
<tr>
<td><em>Bachychiton acerifolius</em></td>
<td>0.58</td>
<td>0.67</td>
<td>1.00</td>
<td>0.75</td>
<td>1.00</td>
<td>0.83</td>
<td>0.81 (0.06)</td>
<td>0.17</td>
<td>0.33</td>
<td>0.67</td>
<td>0.42</td>
<td>0.50</td>
<td>0.40 (0.07)</td>
</tr>
<tr>
<td><em>Cinnamomum camphora</em></td>
<td>0.83</td>
<td>0.83</td>
<td>0.67</td>
<td>0.83</td>
<td>0.75</td>
<td>0.75</td>
<td>0.79 (0.03)</td>
<td>0.58</td>
<td>0.42</td>
<td>0.58</td>
<td>0.58</td>
<td>0.67</td>
<td>0.57 (0.03)</td>
</tr>
<tr>
<td><em>Neolitsea dealbata</em></td>
<td>0.83</td>
<td>0.50</td>
<td>0.33</td>
<td>0.58</td>
<td>0.58</td>
<td>0.42</td>
<td>0.54 (0.07)</td>
<td>0.67</td>
<td>0.50</td>
<td>0.67</td>
<td>0.83</td>
<td>0.67</td>
<td>0.69 (0.05)</td>
</tr>
<tr>
<td><em>Cryptocarya micronoeura</em></td>
<td>0.75</td>
<td>0.08</td>
<td>0.75</td>
<td>0.42</td>
<td>0.25</td>
<td>0.33</td>
<td>0.43 (0.11)</td>
<td>0.75</td>
<td>0.50</td>
<td>0.75</td>
<td>0.58</td>
<td>0.67</td>
<td>0.64 (0.04)</td>
</tr>
<tr>
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<td>0.33</td>
<td>0.33</td>
<td>0.42</td>
<td>0.08</td>
<td>0.33</td>
<td>0.17</td>
<td>0.28 (0.04)</td>
<td>0.83</td>
<td>0.33</td>
<td>0.50</td>
<td>0.50</td>
<td>0.33</td>
<td>0.50 (0.06)</td>
</tr>
<tr>
<td><em>Cryptocarya obovata</em></td>
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<td>0.42</td>
<td>0.58</td>
<td>0.50</td>
<td>0.83</td>
<td>0.67</td>
<td>0.58 (0.06)</td>
<td>0.58</td>
<td>0.42</td>
<td>0.50</td>
<td>0.33</td>
<td>0.50</td>
<td>0.46 (0.03)</td>
</tr>
<tr>
<td><em>Acmena hemilampra</em></td>
<td>0.50</td>
<td>0.50</td>
<td>0.25</td>
<td>0.33</td>
<td>0.33</td>
<td>0.17</td>
<td>0.35 (0.05)</td>
<td>0.67</td>
<td>0.50</td>
<td>0.50</td>
<td>0.58</td>
<td>0.83</td>
<td>0.57 (0.07)</td>
</tr>
<tr>
<td><em>Cryptocarya glaucescens</em></td>
<td>0.42</td>
<td>0.63</td>
<td>0.54</td>
<td>0.33</td>
<td>0.54</td>
<td>0.67</td>
<td>0.62 (0.05)</td>
<td>0.79</td>
<td>0.58</td>
<td>0.50</td>
<td>0.63</td>
<td>0.54</td>
<td>0.60 (0.04)</td>
</tr>
<tr>
<td><em>Podocarpus elatus</em></td>
<td>1.00</td>
<td>0.67</td>
<td>0.83</td>
<td>0.25</td>
<td>0.83</td>
<td>0.17</td>
<td>0.63 (0.13)</td>
<td>1.00</td>
<td>0.58</td>
<td>0.67</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75 (0.05)</td>
</tr>
<tr>
<td><em>Castanosporia alphanidi</em></td>
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<td>0.33</td>
<td>0.33</td>
<td>0.33</td>
<td>0.25</td>
<td>0.08</td>
<td>0.31 (0.06)</td>
<td>0.75</td>
<td>0.58</td>
<td>0.67</td>
<td>0.58</td>
<td>0.25</td>
<td>0.58 (0.06)</td>
</tr>
<tr>
<td><em>Acmena ingens</em></td>
<td>0.08</td>
<td>0.25</td>
<td>0.08</td>
<td>0.25</td>
<td>0.50</td>
<td>0.33</td>
<td>0.25 (0.05)</td>
<td>0.58</td>
<td>0.33</td>
<td>0.08</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42 (0.06)</td>
</tr>
<tr>
<td><em>Castanospermum austral</em></td>
<td>0.92</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.92</td>
<td>0.97 (0.01)</td>
<td>0.83</td>
<td>0.33</td>
<td>0.08</td>
<td>0.58</td>
<td>0.75</td>
<td>0.50 (0.09)</td>
</tr>
<tr>
<td>Small seeds</td>
<td>0.52</td>
<td>0.50</td>
<td>0.50</td>
<td>0.54</td>
<td>0.51</td>
<td>0.49</td>
<td>0.51 (0.01)</td>
<td>0.56</td>
<td>0.47</td>
<td>0.46</td>
<td>0.53</td>
<td>0.52</td>
<td>0.51 (0.01)</td>
</tr>
<tr>
<td>Large seeds</td>
<td>0.54</td>
<td>0.48</td>
<td>0.53</td>
<td>0.38</td>
<td>0.54</td>
<td>0.42</td>
<td>0.48 (0.02)</td>
<td>0.76</td>
<td>0.48</td>
<td>0.48</td>
<td>0.56</td>
<td>0.60</td>
<td>0.56 (0.04)</td>
</tr>
<tr>
<td>All seeds</td>
<td>0.53</td>
<td>0.49</td>
<td>0.52</td>
<td>0.47</td>
<td>0.53</td>
<td>0.45</td>
<td>0.50 (0.01)</td>
<td>0.65</td>
<td>0.47</td>
<td>0.47</td>
<td>0.54</td>
<td>0.56</td>
<td>0.54 (0.02)</td>
</tr>
</tbody>
</table>


Chapter Three supplementary material

S3.1 Seed predator profiles – All common predator taxa and predator functional groups

**Figure S3.1.1** Seed predator profiles (see table 1 for abbreviations). Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Dashed lines represent average destructive interest calculated across all 20 seed species. ‘N’ represents predator not recorded at a seed species.
Figure S3.1.2 Seed predator functional group profiles. Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Dashed lines represent average destructive interest calculated across all 20 seed species.
S3.2 All seed species profiles

Figure S3.2.1 Seed species profiles. Destructive interest calculated as the percent camera days it was recorded where it interfered physically with seeds, averaged across sites. Dashed lines represent average destructive interest calculated across all seven seed predator taxa. Abbreviations for predator taxa are as follows: BCHIN, C. indica; BLEME, L. melanoleuca; BALLA, A. lathami; MMECE, M. cervinipes; MRAFU, R. fuscipes; MRARA, R. rattus; MTRSP, Trichosurus sp.
Chapter Four supplementary material

Table S4.1.1 Spearman’s r correlation matrix of seed traits initially measured in study (N=20). Seed traits in bold are those used in analysis. ‘Fracture resistance’ is the weight required to fracture the seed coat, ‘Width’ is the shortest diameter, ‘E/E weight’ is weight of embryo/endosperm. ‘Wet’ refers to fresh weight, ‘Dry’ refers to weight following over-drying for 24 hours at 60°C, ‘Antifeedant compound’ represents the concentration of compounds containing antifeedant biological activity.

<table>
<thead>
<tr>
<th></th>
<th>Fracture resistance (kg)</th>
<th>Coat thickness (mm)</th>
<th>Relative coat thickness index</th>
<th>Coat wet weight (g)</th>
<th>Coat dry weight (g)</th>
<th>E/E wet weight (g)</th>
<th>E/E dry weight (g)</th>
<th>E/E % of total dry weight</th>
<th>Total wet weight (g)</th>
<th>Total dry weight (g)</th>
<th>Antifeedant compound</th>
<th>Protection index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fracture resistance (kg)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Coat thickness (mm)</td>
<td>0.70**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative coat thickness index</td>
<td>0.57**</td>
<td>0.82**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coat wet weight (g)</td>
<td>-0.18</td>
<td>0.06</td>
<td>0.37</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coat dry weight (g)</td>
<td>-0.15</td>
<td>0.08</td>
<td>0.41</td>
<td>0.97**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E wet weight (g)</td>
<td>-0.05</td>
<td>0.05</td>
<td>0.36</td>
<td>0.91**</td>
<td>0.91**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E dry weight (g)</td>
<td>0.18</td>
<td>0.49*</td>
<td>0.01</td>
<td>-0.31</td>
<td>-0.34</td>
<td>-0.33</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E % of total dry weight</td>
<td>0.17</td>
<td>-0.14</td>
<td>0.27</td>
<td>-0.30</td>
<td>-0.29</td>
<td>0.03</td>
<td>0.12</td>
<td>1.00</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Width (mm)</td>
<td>0.32</td>
<td>0.52</td>
<td>0.13</td>
<td>-0.28</td>
<td>-0.32</td>
<td>-0.26</td>
<td>0.92**</td>
<td>0.09</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total wet weight (g)</td>
<td>-0.07</td>
<td>0.78**</td>
<td>0.39</td>
<td>0.95**</td>
<td>0.95</td>
<td>0.98**</td>
<td>-0.33</td>
<td>-0.07</td>
<td>0.29</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total dry weight (g)</td>
<td>-0.07</td>
<td>0.78**</td>
<td>0.39</td>
<td>0.95**</td>
<td>0.95</td>
<td>0.98**</td>
<td>-0.33</td>
<td>-0.07</td>
<td>0.29</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
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<td>0.31</td>
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<td>0.20</td>
<td>-0.51*</td>
<td>0.15</td>
<td>0.17</td>
<td>0.17</td>
<td>1.00</td>
</tr>
<tr>
<td>Protection index</td>
<td>0.34</td>
<td>0.69**</td>
<td>0.72**</td>
<td>0.25</td>
<td>0.18</td>
<td>0.41</td>
<td>-0.39</td>
<td>0.42*</td>
<td>0.16</td>
<td>0.16</td>
<td>0.79**</td>
<td>1.00</td>
</tr>
<tr>
<td>Interference score</td>
<td>-0.45*</td>
<td>-0.11</td>
<td>-0.06</td>
<td>0.35</td>
<td>0.41*</td>
<td>0.28</td>
<td>-0.09</td>
<td>-0.19</td>
<td>0.08</td>
<td>0.29</td>
<td>0.29</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1Relative coat thickness index is standardised to remove allometric influence of seed size.
2Protection index=Overall score based on fracture resistance, coat thickness and chemical antifeedant score.
Significance values: *P<0.10, *P<0.05, **P<0.01.
S4.2 All compounds isolated from seed species

**Polyphenolics**

**Figure S4.2.1** Molecular structures of polyphenolic compounds isolated from seeds. Name of species that compound was isolated from below structures, with botanical family names, and specific classes of compounds.
**Polyketides**

**Figure S4.2.2** Molecular structures of polyketide compounds isolated from seeds. Name of species that compound was isolated from below structures, with botanical family names, and specific classes of compounds.

**Terpenes**

**Figure S4.2.3** Molecular structures of terpene compounds isolated from seeds. Name of species that compound was isolated from below structures, with botanical family names, and specific classes of compounds.
Alkaloids

**Figure S4.2.4** Molecular structures of alkaloid compounds isolated from seeds. Name of species that compound was isolated from below structures, with botanical family names, and specific classes of compounds.
### Table S4.3.1 Interference scores for each seed species. Calculated as the average damage score of the 10 real seeds and 2 plasticine seeds for each species at each site. Overall: averaged across all 240 combinations of species/site; Cont, frag: averaged across 120 combinations of species/site in continuous forest and fragments respectively. Standard errors in parentheses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Big Scrub</th>
<th>Big Scrub</th>
<th>Big Scrub</th>
<th>Boome-rang Falls</th>
<th>Minyon Falls</th>
<th>Cont av.</th>
<th>Booyong Flora Reserve</th>
<th>Dawes Bush</th>
<th>Davis Scrub</th>
<th>Emery’s Scrub</th>
<th>Johnson’ Nature Reserve</th>
<th>Victoria Park</th>
<th>Frag av.</th>
<th>Overall av.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Melia azedarach</em></td>
<td>0.67</td>
<td>0.42</td>
<td>0.33</td>
<td>0.50</td>
<td>0.58</td>
<td>0.38</td>
<td><strong>0.48 (0.05)</strong></td>
<td>0.67</td>
<td>0.67</td>
<td>0.75</td>
<td>0.75</td>
<td>0.42</td>
<td>0.75</td>
<td><strong>0.67 (0.05)</strong></td>
</tr>
<tr>
<td><em>Denhamia celastroides</em></td>
<td>0.17</td>
<td>0.17</td>
<td>0.50</td>
<td>0.29</td>
<td>0.50</td>
<td>0.33</td>
<td><strong>0.33 (0.06)</strong></td>
<td>0.79</td>
<td>0.58</td>
<td>0.17</td>
<td>0.46</td>
<td>0.33</td>
<td>0.58</td>
<td><strong>0.49 (0.09)</strong></td>
</tr>
<tr>
<td><em>Tabernaemontana pandacaqui</em></td>
<td>0.75</td>
<td>1.00</td>
<td>0.63</td>
<td>0.92</td>
<td>0.50</td>
<td>0.83</td>
<td><strong>0.77 (0.08)</strong></td>
<td>0.67</td>
<td>0.33</td>
<td>0.42</td>
<td>0.42</td>
<td>0.42</td>
<td>0.45</td>
<td><strong>0.45 (0.05)</strong></td>
</tr>
<tr>
<td><em>Eupomatia laurina</em></td>
<td>0.54</td>
<td>0.58</td>
<td>0.50</td>
<td>0.42</td>
<td>0.17</td>
<td>0.42</td>
<td><strong>0.44 (0.06)</strong></td>
<td>0.33</td>
<td>0.42</td>
<td>0.58</td>
<td>0.42</td>
<td>0.50</td>
<td>0.58</td>
<td><strong>0.47 (0.04)</strong></td>
</tr>
<tr>
<td><em>Guioa semiglaucu</em></td>
<td>0.04</td>
<td>0.33</td>
<td>0.29</td>
<td>0.46</td>
<td>0.42</td>
<td>0.42</td>
<td><strong>0.33 (0.06)</strong></td>
<td>0.50</td>
<td>1.00</td>
<td>0.08</td>
<td>0.67</td>
<td>0.42</td>
<td>0.38</td>
<td><strong>0.51 (0.13)</strong></td>
</tr>
<tr>
<td><em>Mallotus philippicens</em></td>
<td>0.83</td>
<td>0.58</td>
<td>0.67</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td><strong>0.60 (0.05)</strong></td>
<td>0.92</td>
<td>0.83</td>
<td>1.00</td>
<td>1.00</td>
<td>0.92</td>
<td>1.00</td>
<td><strong>0.92 (0.04)</strong></td>
</tr>
<tr>
<td><em>Atractocarpus chartaceus</em></td>
<td>0.75</td>
<td>0.58</td>
<td>0.21</td>
<td>0.58</td>
<td>0.63</td>
<td>0.54</td>
<td><strong>0.55 (0.07)</strong></td>
<td>0.54</td>
<td>0.33</td>
<td>0.25</td>
<td>0.42</td>
<td>0.63</td>
<td>0.42</td>
<td><strong>0.43 (0.06)</strong></td>
</tr>
<tr>
<td><em>Ehretia acuminata</em></td>
<td>0.08</td>
<td>0.10</td>
<td>0.25</td>
<td>0.29</td>
<td>0.13</td>
<td>0.17</td>
<td><strong>0.17 (0.03)</strong></td>
<td>0.35</td>
<td>0.13</td>
<td>0.17</td>
<td>0.19</td>
<td>0.31</td>
<td>0.42</td>
<td><strong>0.26 (0.05)</strong></td>
</tr>
<tr>
<td><em>Brachychiton acerifolius</em></td>
<td>0.58</td>
<td>0.63</td>
<td>0.92</td>
<td>0.71</td>
<td>0.92</td>
<td>0.75</td>
<td><strong>0.75 (0.06)</strong></td>
<td>0.17</td>
<td>0.33</td>
<td>0.67</td>
<td>0.42</td>
<td>0.50</td>
<td>0.33</td>
<td><strong>0.40 (0.07)</strong></td>
</tr>
<tr>
<td><em>Cinnamomum camphora</em></td>
<td>0.83</td>
<td>0.83</td>
<td>0.63</td>
<td>0.79</td>
<td>0.79</td>
<td>0.75</td>
<td><strong>0.77 (0.03)</strong></td>
<td>0.58</td>
<td>0.42</td>
<td>0.58</td>
<td>0.58</td>
<td>0.63</td>
<td>0.54</td>
<td><strong>0.56 (0.03)</strong></td>
</tr>
<tr>
<td><em>Neolitsea dealbata</em></td>
<td>0.79</td>
<td>0.46</td>
<td>0.33</td>
<td>0.58</td>
<td>0.54</td>
<td>0.38</td>
<td><strong>0.51 (0.07)</strong></td>
<td>0.58</td>
<td>0.50</td>
<td>0.63</td>
<td>0.79</td>
<td>0.83</td>
<td>0.58</td>
<td><strong>0.65 (0.05)</strong></td>
</tr>
<tr>
<td><em>Cryptocarya microneura</em></td>
<td>0.67</td>
<td>0.08</td>
<td>0.75</td>
<td>0.42</td>
<td>0.17</td>
<td>0.33</td>
<td><strong>0.40 (0.11)</strong></td>
<td>0.71</td>
<td>0.46</td>
<td>0.67</td>
<td>0.58</td>
<td>0.50</td>
<td>0.63</td>
<td><strong>0.59 (0.04)</strong></td>
</tr>
<tr>
<td><em>Wilkeia huegeliana</em></td>
<td>0.25</td>
<td>0.29</td>
<td>0.33</td>
<td>0.08</td>
<td>0.29</td>
<td>0.13</td>
<td><strong>0.23 (0.04)</strong></td>
<td>0.71</td>
<td>0.33</td>
<td>0.50</td>
<td>0.50</td>
<td>0.33</td>
<td>0.50</td>
<td><strong>0.48 (0.06)</strong></td>
</tr>
<tr>
<td><em>Cryptocarya obovata</em></td>
<td>0.42</td>
<td>0.42</td>
<td>0.54</td>
<td>0.50</td>
<td>0.79</td>
<td>0.67</td>
<td><strong>0.56 (0.06)</strong></td>
<td>0.50</td>
<td>0.42</td>
<td>0.42</td>
<td>0.33</td>
<td>0.33</td>
<td>0.42</td>
<td><strong>0.40 (0.03)</strong></td>
</tr>
<tr>
<td><em>Acmena hemilampra</em></td>
<td>0.41</td>
<td>0.42</td>
<td>0.25</td>
<td>0.29</td>
<td>0.21</td>
<td>0.13</td>
<td><strong>0.28 (0.05)</strong></td>
<td>0.58</td>
<td>0.50</td>
<td>0.50</td>
<td>0.58</td>
<td>0.79</td>
<td>0.29</td>
<td><strong>0.54 (0.07)</strong></td>
</tr>
<tr>
<td><em>Cryptocarya glaucescens</em></td>
<td>0.33</td>
<td>0.58</td>
<td>0.52</td>
<td>0.33</td>
<td>0.52</td>
<td>0.63</td>
<td><strong>0.49 (0.05)</strong></td>
<td>0.75</td>
<td>0.48</td>
<td>0.50</td>
<td>0.58</td>
<td>0.60</td>
<td>0.46</td>
<td><strong>0.56 (0.04)</strong></td>
</tr>
<tr>
<td><em>Podocarpus elatus</em></td>
<td>0.92</td>
<td>0.58</td>
<td>0.79</td>
<td>0.25</td>
<td>0.79</td>
<td>0.17</td>
<td><strong>0.58 (0.13)</strong></td>
<td>0.92</td>
<td>0.58</td>
<td>0.63</td>
<td>0.71</td>
<td>0.75</td>
<td>0.75</td>
<td><strong>0.72 (0.05)</strong></td>
</tr>
<tr>
<td><em>Castanospora alpandii</em></td>
<td>0.50</td>
<td>0.29</td>
<td>0.33</td>
<td>0.33</td>
<td>0.17</td>
<td>0.08</td>
<td><strong>0.28 (0.06)</strong></td>
<td>0.58</td>
<td>0.46</td>
<td>0.54</td>
<td>0.58</td>
<td>0.46</td>
<td>0.21</td>
<td><strong>0.47 (0.06)</strong></td>
</tr>
<tr>
<td><em>Acmena ingens</em></td>
<td>0.08</td>
<td>0.25</td>
<td>0.08</td>
<td>0.17</td>
<td>0.38</td>
<td>0.21</td>
<td><strong>0.19 (0.05)</strong></td>
<td>0.50</td>
<td>0.17</td>
<td>0.08</td>
<td>0.42</td>
<td>0.33</td>
<td>0.38</td>
<td><strong>0.31 (0.06)</strong></td>
</tr>
<tr>
<td><em>Castanospermum australe</em></td>
<td>0.83</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.92</td>
<td>0.88</td>
<td><strong>0.90 (0.01)</strong></td>
<td>0.58</td>
<td>0.25</td>
<td>0.08</td>
<td>0.54</td>
<td>0.71</td>
<td>0.42</td>
<td><strong>0.43 (0.09)</strong></td>
</tr>
<tr>
<td>Small seeds</td>
<td>0.51</td>
<td>0.48</td>
<td>0.46</td>
<td>0.53</td>
<td>0.48</td>
<td>0.47</td>
<td><strong>0.49 (0.01)</strong></td>
<td>0.54</td>
<td>0.47</td>
<td>0.45</td>
<td>0.52</td>
<td>0.50</td>
<td>0.54</td>
<td><strong>0.51 (0.01)</strong></td>
</tr>
<tr>
<td>Large seeds</td>
<td>0.47</td>
<td>0.44</td>
<td>0.50</td>
<td>0.36</td>
<td>0.48</td>
<td>0.38</td>
<td><strong>0.44 (0.02)</strong></td>
<td>0.66</td>
<td>0.41</td>
<td>0.44</td>
<td>0.54</td>
<td>0.54</td>
<td>0.45</td>
<td><strong>0.51 (0.04)</strong></td>
</tr>
<tr>
<td>All seeds</td>
<td>0.49</td>
<td>0.46</td>
<td>0.48</td>
<td>0.45</td>
<td>0.48</td>
<td>0.43</td>
<td><strong>0.47 (0.01)</strong></td>
<td>0.59</td>
<td>0.45</td>
<td>0.45</td>
<td>0.53</td>
<td>0.52</td>
<td>0.50</td>
<td><strong>0.51 (0.02)</strong></td>
</tr>
</tbody>
</table>
Chapter Five supplementary material

S5.1 Exploration of between-season variability in duplicate species

Because field data collection was conducted across two seasons, two duplicate seed species were used throughout the second season field year to enable a test of between-year variability.

In addition to the ten species used for the first time in year two, the seeds of the two duplicates (Cryptocarya glaucescens and Ehretia acuminata) were established across all field sites, using methods as described in Chapter 6. To test for statistically significant differences in interference scores between years, a nested ANOVA using sites as subjects was used to assess the effect of site type (two) and year (two) on interference scores. ANOVA was carried out using RStudio version 3.1.2.

Table S5.1.1. Results of nested ANOVA analysis testing effects of site type (N=2) and year (N=2) on interference scores of duplicate species (C. glaucescens and E. acuminata).

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glaucescens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site type</td>
<td>1</td>
<td>0.62</td>
<td>0.60</td>
<td>1</td>
<td>1.30</td>
<td>0.28</td>
</tr>
<tr>
<td>Year</td>
<td>1</td>
<td>0.25</td>
<td>0.63</td>
<td>1</td>
<td>1.08</td>
<td>0.32</td>
</tr>
<tr>
<td>Site (site type)</td>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Site type x year</td>
<td>1</td>
<td>0.002</td>
<td>0.96</td>
<td>1</td>
<td>0.79</td>
<td>0.39</td>
</tr>
<tr>
<td>Site (site type) x year</td>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure S5.1.1 Between-year and site type variability in interference scores of duplicate species. Circles represent interference scores in continuous forest or fragments (N=6 sites in each). Lines represent mean values averaged across sites for each site type in each season (N=6 sites in each).
### S5.2 Rates of seed removal recorded from seed stations (individual sites)

Table S5.2.1 Proportion of seeds removed for each seed species. Calculated as the proportion of seeds removed from each combination of seed species/study site (12 seeds for each combination, 24 seeds for each combination of duplicate species). Overall: averaged across all 240 combinations of species/site; Cont, frag: averaged across 120 combinations of species/site in continuous forest and fragments respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Continuous forest</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Big Scrub 1</td>
<td>Big Scrub 2</td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>0.67</td>
<td>0.42</td>
</tr>
<tr>
<td>Denhamia celastrodes</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Tabernaemontana pandacaqui</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td>Eupomatia laurina</td>
<td>0.50</td>
<td>0.58</td>
</tr>
<tr>
<td>Guioa semiglaucu</td>
<td>0.00</td>
<td>0.33</td>
</tr>
<tr>
<td>Mallotus philippensis</td>
<td>0.83</td>
<td>0.58</td>
</tr>
<tr>
<td>Atractocarpus chartaceus</td>
<td>0.75</td>
<td>0.58</td>
</tr>
<tr>
<td>Efreitia acuminata</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Brachychiton acerifolius</td>
<td>0.58</td>
<td>0.58</td>
</tr>
<tr>
<td>Cinnamomum camphora</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>Neolitsea dealbata</td>
<td>0.75</td>
<td>0.42</td>
</tr>
<tr>
<td>Cryptocarya microneura</td>
<td>0.58</td>
<td>0.08</td>
</tr>
<tr>
<td>Wilkea huegeliana</td>
<td>0.17</td>
<td>0.25</td>
</tr>
<tr>
<td>Cryptocarya obovata</td>
<td>0.33</td>
<td>0.42</td>
</tr>
<tr>
<td>Acmena hemilandra</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>Cryptocarya glaucescens</td>
<td>0.25</td>
<td>0.54</td>
</tr>
<tr>
<td>Podocarpus elatus</td>
<td>0.83</td>
<td>0.50</td>
</tr>
<tr>
<td>Castanopsis alpandii</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>Acmena ingens</td>
<td>0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>Castanospermum australe</td>
<td>0.75</td>
<td>0.83</td>
</tr>
</tbody>
</table>

| Small seeds                    | 0.50              | 0.47       | 0.43       | 0.51       | 0.45         | 0.45         | 0.47      | 0.52         | 0.47       | 0.45       | 0.51       | 0.49       | 0.52       | 0.50           | 0.48               |
| Large seeds                    | 0.41              | 0.40       | 0.48       | 0.34       | 0.41         | 0.35         | 0.40      | 0.59         | 0.35       | 0.38       | 0.53       | 0.49       | 0.39       | 0.46           | 0.43               |
| All seeds                      | 0.46              | 0.44       | 0.45       | 0.44       | 0.43         | 0.41         | 0.44      | 0.55         | 0.42       | 0.42       | 0.52       | 0.49       | 0.46       | 0.48           | 0.46               |
### S5.3 Common vertebrate seed predator abundance

Vertebrate seed predators and their behaviour were recorded in Chapter 3. One motion-activated infra-red video camera trap was focused on the seeds at one station for each seed species at each site (i.e. a total of 22 camera sessions with seeds at each site). Additionally, six camera traps were established at stations without seeds at each site, giving an overall total of 28 five-day camera sessions at each site (140 camera days, comprising 110 days at stations with seeds and 30 days at stations without seeds). Most events that activated the cameras’ motion sensors were movements of vertebrate animals. To provide an index of vertebrate abundance at each site, I calculated the percentage of total camera days (140/site) during which each vertebrate taxon was present. This value was then averaged across all 12 sites, and also separately in fragments and continuous forest sites (N=6 for each).

Seven common (present at six or more sites) vertebrate predator taxa were identified, comprising three small mammal rodents (*Rattus fuscipes, Rattus rattus, Melomys cervinipes*), one large mammal possum (*Trichosurus* sp.), one large bird (*Alectura lathami*), and two small birds (*Chalcophaps indica* and *Leucosarcia melanoleuca*).

**Table S5.3.1** Occupancy rates of common predator taxa. Calculated as the percentage of total camera days (140/site) during which a given taxon was present. Values averaged across six sites in fragments and six in continuous forest.

<table>
<thead>
<tr>
<th>Site type</th>
<th>Site name</th>
<th><em>A. lathami</em></th>
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<th><em>L. melanoleuca cervinipes</em></th>
<th><em>M. fuscipes</em></th>
<th><em>R. rattus</em></th>
<th><em>Trichosurus</em> sp.</th>
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