Assessing frequency-dependent seed size selection: a field experiment

JUAN L. CELIS-DIEZ, RAMIRO O. BUSTAMANTE* and RODRIGO A. VÁSQUEZ

Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Seed size is a life history attribute that affects the probability of seed predation, and therefore affects plant fitness. Compared with smaller seeds, those with large size should be more attractive to predators, as they constitute a more profitable food item because of higher energetic and/or nutrient content. However, predator preferences may be frequency-dependent in the sense that they may be modulated by the relative abundance of alternative seeds of different sizes. We set up a field experiment to evaluate frequency-dependent seed predation using seeds of *Cryptocarya alba* (Lauraceae), at La Campana National Park in central Chile. Predators (rodents and birds) preferentially consumed large seeds in an antiapostatic manner. These selective responses were maintained throughout the experiment and seed selection by predators was not affected by previous seed consumption. Our results suggest that (a) large seeds are very profitable food items actively sought by seed predators even at low relative abundance, (b) seed selection is expressed in a short time scale and (c) seed predators, by consuming large seeds consistently, have the potential to modify significantly the quality of plant progeny. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **81**, 307–312.

ADDITIONAL KEYWORDS: antiapostatic selection – *Cryptocarya alba* – La Campana National Park – Mediterranean shrubland – seed predation.

INTRODUCTION

Seed size is a life history trait consistently affecting plant fitness: generally, larger seeds are more successful than are smaller seeds in terms of seedling recruitment and/or plant competition (Harper, 1977; Fenner, 1985; Silvertown, 1989). Intraspecifically, seed size varies considerably both between and within populations (Winn, 1988; Silvertown, 1989; Mojonnier, 1998), and explanations for such variability have often relied upon plastic responses of the parental plants to environmental conditions (Silvertown, 1989). It is expected that larger seeds are preferred by seed predators because of their higher profitability in energetic and/or nutrient content (Molau, Eriksen & Teilmann, 1989; Vander Wall, 1995; Moegenburg, 1996; Brewer, 2001). The preference of seed predators depends also on the relative abundance of alternative phenotypes. In other words, seed selection can be frequency-dependent (Greenwood, 1985; Allen, 1988). It can be apostatic, when predators concentrate on large seeds when they are common in the environment, or it can be antiapostatic, when larger seeds are disproportionately eaten when they are scarce. Seed selection may also be frequency-independent, for example when large seed are preferred irrespective of their relative abundance.

Frequency-dependent prey selection can be particularly useful for understanding the mechanisms underlying predator–prey interactions and the evolutionary implications of such responses (Allen, 1988; Endler, 1988; Rodríguez-Gironés & Vásquez, 2002). However, this approach has rarely been used in studies on seed predation. The few studies of this sort have been conducted under laboratory or seminatural conditions, using commercial or artificial seeds (Greenwood, 1985; Greenwood, Blow & Thomas, 1984a; Greenwood, Johnston & Thomas, 1984b; Allen, 1988; Church, Jowers & Allen, 1997; Weale *et al.*, 2000). This approach, although instructive in elucidating the mechanism involved in prey selection, lacks reality as it is not always possible to extrapo-

*Corresponding author. E-mail: rbustama@uchile.cl

late the results to natural conditions. We examined frequency-dependent seed size selection of *Cryptocarya alba* (Mol.) Looser, a common endemic tree of the Mediterranean region of central Chile. We addressed the following questions: (i) do seed predators express frequency-dependent seed size selection? (ii) Are such selective predatory responses long-lasting (a question that is critical to future attempts to detect frequency-dependent seed size selection)? (iii) How is a given pattern of frequency-dependent seed predation affected by previous seed consumption? This last issue reflects a fundamental methodological difficulty in empirical food preference and predation studies: the fact that predators themselves may in turn modify the prey frequencies and therefore affect their own selective responses in later consumption (see e.g. Vásquez & Kacelnik, 2000).

METHODS

This study was carried out at La Campana National Park (32°57′S, 71°08′W), in the coastal range of Central Chile. The vegetation is characterized by Mediterranean shrubland where tree species such as *C. alba, Peumus boldus* Mol.*, Quillaja saponaria* Mol. and *Litrhaea caustica* (Mol.) H. et A. are dominant (Villaseñor & Serey, 1980/81). *C. alba* is a 4–30-m tall, shade-tolerant evergreen tree (Armesto & Pickett, 1985), living on south-facing slopes and in humid ravines (Armesto & Martínez, 1978). Its fruits are red to pink one-seeded drupes (Hoffmann, 1998) that vary in seed size both within parental trees and at the population level (seed mass ranges from 0.1 to 3.3 g; see Chacón & Bustamante, 2001). The main seed preda-

tors are rodents (including *Abrothrix longipilis* (Water)*, A. olivaceus* (Water)*, Oligoryzomis longicaudatus* (Bennett), and *Phyllotis darwini* (Water)) and birds (such as *Lofortyx californica* (Shaw) and *Diuca diuca* (Mol.)) (see Bustamante & Vásquez, 1995).

We collected 2000 seeds from 20 individual trees (100 randomly selected seeds per tree). For the purposes of this study, we defined a seed as the whole dispersal unit, i.e. the seed plus the pericarp that covers it. We measured the size of a seed by its fresh weight. The size distribution of collected seeds is shown in Figure 1. We operationally defined a 'small seed' as one included within the lower 15% of the distribution (range, 0.1–0.61 g), and a 'large seed' as one included within the higher 15% of the population distribution (1.61–2.8 g).

During July and August 2000 we set up a field experiment comprising five treatments that varied in the relative proportion of large seeds to small seeds: 0.1, 0.25, 0.50, 0.75 and 0.9. We used five replicates per treatment at the same seed density (50 seeds m^{-2}; see Bustamante & Simonetti, 2000). Replicates were arranged in a 60×60-m grid with each station located 15 m apart, making a total of 25 experimental stations. The proportion of large to small seeds at each station was selected at random with the restriction of not allowing three equal proportions being at neighbouring stations. We observed the number of removed seeds after 7, 14 and 21 days; After 2 weeks, no significant seed removal was observed (Fig. 2).

In order to answer the three questions addressed in this study, we conducted the following analysis.
a) Seed survival using log rank analysis to compare the fate of two cohorts of seeds (Lee, Desu & Gehan,
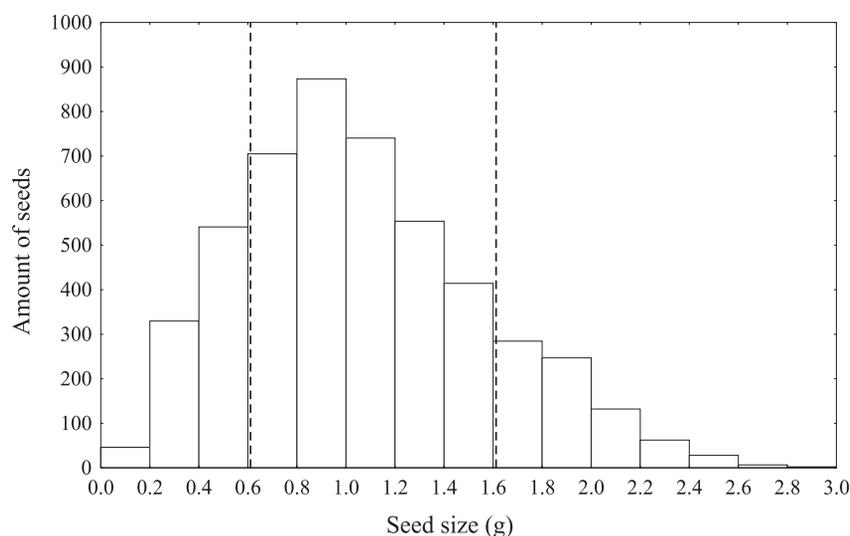

Figure 1. Seed size frequency distribution from 20 parental trees (100 seeds per individual) in La Campana National Park during 2000. Dashed lines are 15% and 85% percentiles.
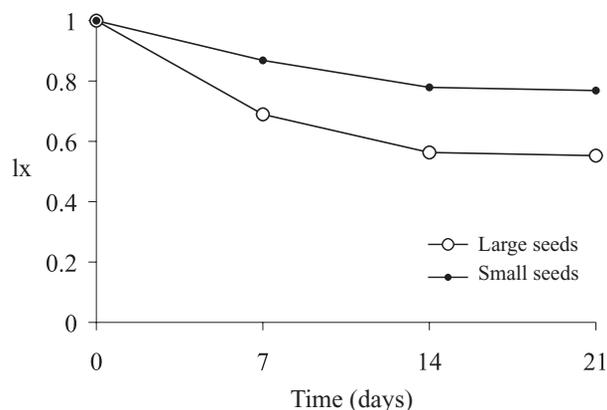
Figure 2. Seed survival (lx) of large and small seeds at equal relative abundance in *Cryptocarya alba*. Data points have no error values (discussed in Methods).

1975), in this case large vs. small seeds. For this analysis, we used the data obtained from the treatment 0.5 : 0.5 large : small seeds to correct for frequency-dependence. As seed survival analysis registers the fate of seeds belonging to only one cohort, no confidence intervals may be obtained from this method.

b) We compared seed preferences for large seeds between the periods (i) 0–7 days and (ii) 0–14 days through an ANCOVA; data satisfied assumptions of the test.

c) We compared seed preferences for large seeds between the periods (i) 0–7 days and (ii) 7–14 days.

For points (b) and (c), we performed an analysis of frequency-dependent selection following the model of Greenwood & Elton (1979):

$$\frac{e_L}{e_S} = \left(V\,\frac{A_L}{A_S}\right)^b$$

Where, e_L / e_S is the ratio of the number of large/ number of small seeds consumed by predators and A_L / A_S is the ratio of the number of large/number of small seeds present in the environment. The parameter b is a measure of the frequency-dependence. If $b > 1$, then selection is apostatic; if $b < 1$, then selection is antiapostatic; if $b = 1$, then selection is frequency-independent. The parameter V evaluates the bias to one specific kind of prey. In our case, if $V > 1$, then there is a bias towards large seeds; if $V < 1$, then there is a bias towards small seeds; if $V = 1$, then there is no bias. To test the statistical significance of b, we conducted log–log regression analysis (Greenwood & Elton, 1979). The significance of the parameter V was tested using $t = b \log V / \mathrm{SE}$, with $n-2$ degrees of freedom.

RESULTS

By the end of the experiment (21 days), the proportion of seed survival (mean ± SE) was 0.50 ± 0.08 ($n = 5$) in large seeds and 0.77 ± 0.11 ($n = 5$) in small seeds. Seed survival differed significantly with seed size (log rank L = −8.36; $P < 0.0001$; see Fig. 2).

We observed an antiapostatic pattern of seed size predation on the 7th day of the period from day 0 to 7 ($b = 0.66$; $t = -2.06$; d.f. = 1, 12; $P < 0.05$; Fig. 3A), with $b \log V$ significantly higher than 0 ($t = 3.10$; d.f. = 12; $P = 0.009$). Similarly, antiapostatic selection was also detected on day 14 (predation period from day 0 to 14) ($b = 0.61$; $t = -2.52$; d.f. = 1, 15; $P < 0.02$; Fig. 3B), again with $b \log V$ significantly higher than 0 ($t = 3.25$; d.f. = 15; $P = 0.005$). No differences were detected between the curves obtained for the period from day 0 to day 7 and the period from day 0 to day 14 (ANCOVA, $F_{1,27} = 0.434$; $P = 0.836$).

To analyse how frequency-dependent seed selection was affected by previous seed predation we considered the seeds remaining on day 7 as the initial distribution for the predation period from day 7–14. By the end of this period, seed predators had reduced the availability of large seeds available in the field (Fig. 4). This reduction was higher when the frequency of large seeds was low. In fact, by day 21 the treatment with proportion 0.1 of large seeds was reduced by 63% relative to day 0 while the treatment with proportion 0.9 of large seeds was constant throughout the experiment (Fig. 4). Despite these results, for the period from day 7 to 14, we detected antiapostatic seed selection ($b = 0.59$; $t = -3.25$; d.f. = 7; $P = 0.02$; see Fig. 3C), with $b \log V$ not significantly higher than 0 ($t = 1.56$; d.f. = 7; $P = 0.16$); this result was not significantly different from that for the period from day 0 to 7 (ANCOVA $F_{1,20} = 0.36$; $P = 0.56$; Fig. 3A,B).

DISCUSSION

The results of frequency-dependent prey selection can be interpreted in the context of optimal foraging theory (Hubbard *et al.*, 1982). If a seed predator is an optimal forager, then it will consume large seeds in an apostatic manner. That is, in order to minimize searching time and/or to maximize energetic intake, seed predators will consume larger seeds at a disproportionately lower rate when they are scarce and at a disproportionately higher rate when they become abundant (Hubbard *et al.*, 1982; Greenwood, 1984; Allen, 1988). However, the detection of persistent antiapostatic seed size selection in our study differs significantly from the afore-mentioned theoretical expectation derived from optimal foraging theory and merits explanation. First, individually based explana-
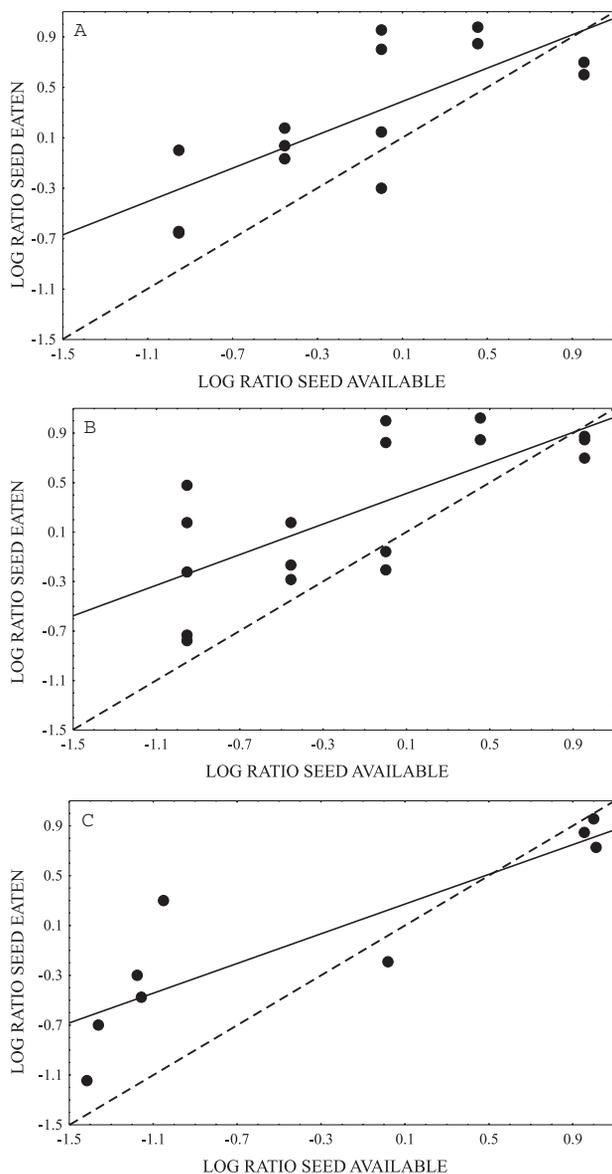
Figure 4. Reduction in the proportion of large seeds available due to seed consumption in *Cryptocarya alba*. Data points have no error values (see Methods).

Figure 3. Seed consumption in *Cryptocarya alba* as a function of the relative abundance of large seeds available in the field; five treatments: 0.1, 0.25, 0.5, 0.75 and 0.9, at the same seed density (50 seeds m^{-2}). Seed consumption from (A) day 0 to day 7 ($n = 14$), (B) day 0 to day 14 ($n = 17$), and (C) day 7 to day 14 ($n = 9$; considering the seeds remaining at day 7 as the initial distribution for the period day 7–14). Dotted line represents no selective response ($b = 1$, frequency-independent selection *sensu* Greenwood, 1985).

avian and mammalian predating species (Busta-mante, 1996). Second, it is reasonable to assume that certain types or species of predators always take large seeds while others always take small seeds. This could lead to antiapostatic seed size selection (Allen & Anderson, 1984; Sherratt & Macdougall, 1995), for instance if large predators are more abundant compared with smaller ones. Third, it is possible that when large seeds are scarce they may be easily detected by seed predators because they become conspicuous against a background of the more frequent small seeds (Landeau & Terborgh, 1986; Allen, Raison & Weale, 1998). Another study assessed this hypothesis (Celis-Diez, 2002).

The early detection (within 7 days) of antiapostatic seed size selection and the maintenance of this selective consumption without changes over the rest of the experimental period (Fig. 4), support the classic protocol of short-term experiments as being adequate to examine selective population responses by seed predators. In our study, it is possible that seed predators rapidly depressed the experimental patches to a threshold level beyond which they became unattractive and so the predators switched to alternative resources in the environment. In our study site there are abundant alternative resources, such as seeds of *Persea lingue* Nees (Lauraceae) and *Sophora macrocarpa* J. E. Sm. (Papilionaceae), that coexist on the floor of the forest with seeds of *C. alba* (Bustamante, 1996). On the other hand, the short period of consumption observed may not have allowed time for the seeds to germinate and recruit as seedlings; seeds of *C. alba* begin to germinate after a fortnight (Bustamante, Simonetti & Mella, 1992) and therefore may have escaped seed predation. Thus, we may have underestimated the intensity of seed predation.

tions related to optimal foraging theory must be considered with caution. Our seed predators were a guild of predators that probably differ in foraging behaviour and therefore might use a range of different cues beyond seed size to select prey optimally. Certainly, the *C. alba* seeds in our study were exposed to diverse
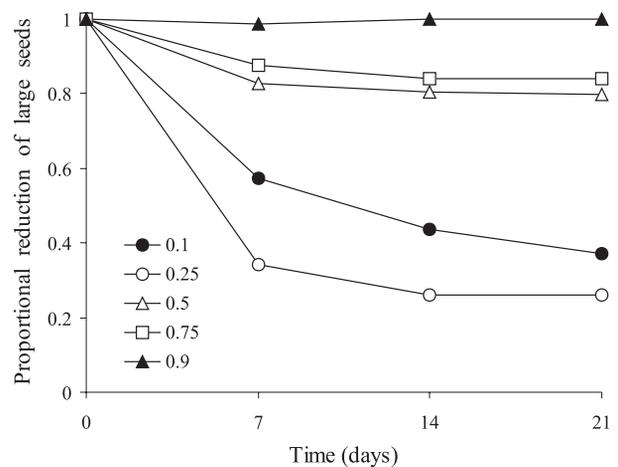
In studies of predation, several researchers have noted the problem of changes in the initial distribution or abundance of prey caused by the predator itself (e.g. the predator's functional response; Hassell, 1978). Our results show that we could have assessed frequency dependence at any time between day 7 and day 21, and the overall results would have remained unaffected. Although we did not assess finer-scale changes in prey distribution (between days 0 and 7), we are confident that the predator guild produced antiapostatic selection on large seeds of *C. alba* throughout the experiment.

Changes in frequency-dependent seed size selection through time should result when seed predators modify the relative abundance of their preferred seeds. In our experiment, large seeds were consistently consumed, suggesting that they are an attractive resource for predators, probably due to their higher energetic content and/or lower chemical defences compared with small seeds (Janzen, 1969; Geritz, 1998), and therefore they compensate for the extra searching effort required to consume an infrequent type of food. Obviously, this possibility demands assessment of benefit/cost ratios.

To what extent are the detected patterns of frequency-dependent seed size predation a function of seed biomass and/or seed numbers? We carried out the same statistical analysis transforming our data from seed numbers to their equivalent seed biomass and the results remained unchanged (analysis not shown). This issue is pertinent to further work, particularly when large and small seeds are numerically identical but clearly different in total biomass.

A consistent preference for large seeds implies that the predator will modify the seed size distribution. By favouring the 'escape' of smaller seeds, seed predators may exert directional selection with the potential to reduce the mean size of the surviving seed population (Winn, 1988). It is known that a higher proportion of larger seeds germinate and they produce more vigorous seedlings with higher competitive abilities relative to smaller seeds (Harper, 1977; Winn, 1988; Silvertown, 1989; see also Chacón & Bustamante, 2001; Chacón, Bustamante & Henríquez, 1998, for examples in *C. alba*). Therefore, seed predators, by consuming preferentially larger seeds, are in turn reducing the quality of plant offspring available for further plant regeneration. This issue requires further theoretical and empirical research.

ACKNOWLEDGEMENTS

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