Functional Ecology 2006
20, 1070–1079

Foraging by fearful frugivores: combined effect of fruit ripening and predation risk

J. M. FEDRIANI† and R. BOULAY

Estación Biológica de Doñana (CSIC), Avda Maria Luisa s/n, Sevilla 41013, Spain

Summary

1. Plant defensive compounds and predation risk are main determinants of herbivore foraging, though empirical studies have seldom measured the combined effects of these two factors. By considering the interaction between the herb *Helleborus foetidus* and its main fruit and seed predator, the Wood Mouse *Apodemus sylvaticus*, we evaluated whether the defensive role against seed predators of compounds present in *H. foetidus* unripe fruits holds across a micro-landscape that differs in foraging costs (i.e. predation risk).

2. First, we used standardized food patches that simulated fruiting *H. foetidus* plants to ascertain fruit preferences of captive mice. Then, by means of field experiments, we assessed the combined effects of fruit ripening and predation risk on foraging by free-ranging mice.

3. Captive mice avoided plants with unripe fruit and avoided consuming unripe fruits within a particular plant. Free-ranging mice also avoided unripe fruits in safe microhabitats (rocky substrate), but not in risky microhabitats (bare ground) where few fruits were consumed. This unexpected result may be driven by predation risk experienced by mice foraging on *H. foetidus* fruits, and/or plant defensive compounds acting in a dose-dependent manner.

4. Frugivorous mice responded to both chemical defences present in unripe *H. foetidus* fruits as well as to predation cost though such response was sequential. Plant defence compounds appeared to play a part in mouse foraging only after mice selected low predation risk microhabitats.

5. Our study indicates that both digestive and ecological factors influence foraging decisions, which in turn affects pressures exerted by herbivores on plant populations.

Key-words: frugivory, fruit ripening, plant chemical defences, predation risk, seed depredation

Functional Ecology (2006) **20**, 1070–1079
doi: 10.1111/j.1365-2435.2006.01199.x

Introduction

An understanding of the effects of plant defensive compounds on a forager's food choice requires consideration of both the defensive compounds and the environment to which the forager is exposed (Karasov & Diamond 1988; Bozinovic & Martínez del Río 1996; Whelan & Brown 2005). However, few studies have accounted for both of these factors simultaneously impinging on animal foraging (Schmidt, Brown & Morgan 1998; Schmidt 2000; Hochman & Kotler 2006). In particular, foraging by frugivores has rarely been studied within the framework of foraging theory (Whelan *et al*. 1998), even though unripe fruits of many plants comprise a myriad of compounds that act as defences against frugivore seed predators (Cipollini & Levey 1997; Herrera 2002). Moreover, Schmidt (2000) showed that predation risk and plant defensive compounds can interact in a nonintuitive manner to determine patterns of herbivory. Because the defence compounds in fruits often gradually disappear during ripening, most frugivores presumably prefer ripe over unripe fruits (Sumner & Mollon 2000; Schaefer, Schmidt & Winkler 2003, Schaefer, Schmidt & Levey 2004; Dudley 2004). However, it is unknown whether the defensive properties of unripe fruits hold across a landscape that differs in foraging costs such as predation risk. In other words, what is the relative importance of fruit defensive compounds and risk of depredation in determining foraging by frugivores?

Animal foraging decisions often take place at several spatial scales (i.e. from local to regional) and the pattern and extent of resource selection is frequently incongruent across these scales (Morris 1992; Morgan, Brown & Thorson 1997). Foraging decisions by

†Author to whom correspondence should be addressed.
E-mail: fedriani@ebd.csic.es

frugivores can occur at a variety of levels, including the plant level within a population or the fruit level within a fruit crop (Sallabanks 1993; Jordano 1995). The recognition of the level(s) that a forager distinguishes food items that differ in quality is critical in understanding its diet choice strategy (Brown & Mitchell 1989; Hochman & Kotler 2006). In addition, the assessment of the pattern of selection by frugivores across different levels (i.e. concordant vs. discordant) may indicate a frugivore's ability to affect plant population dynamics and, ultimately, to exert selection pressures on plant traits (e.g. fruiting phenology and synchrony; secondary compounds; Cipollini & Levey 1997; Herrera 2002). For example, if inconsistencies in multilevel choices by frugivores are commonplace, this could explain why, despite comprehensive research efforts, so few (if any) plant–frugivore systems appear tightly coevolved (Herrera 2002).

The amount of food remaining in a patch when a forager leaves (the giving-up densities; GUDs) is largely affected by the rate of finding food items within the patch, and a variety of direct and indirect foraging costs that include metabolic, predation and missed opportunity costs (Brown 1988). Given the extreme heterogeneity in abundance, size, and ripening stage of fruits within fruiting plants, natural GUDs by frugivores are difficult to obtain (Fedriani & Manzaneda 2005). However, even in these cases, the GUD conceptual framework (Brown & Kotler 2004) can be extended to the case in which food items contain chemical defences (Schmidt 2000), and thus potentially help us to estimate a frugivore's costs and benefits of foraging. In a study of the interaction between the herb *Helleborus foetidus* L. and its main fruit and seed predator in the Iberian Peninsula, the Wood Mouse (*Apodemus sylvaticus* L.), it was found that fruit predation on plants located on rocky substrates was much higher than on plants located on neighbouring open areas (Fedriani 2005). This result is likely associated with the rocky substrates providing mice with shelter from predators, and is consistent with results of other studies (Brown, Morgan & Dow 1992; Kotler *et al*. 2001). Unpublished data suggest that the selection of *H. foetidus* fruits by mice may also be associated with the fruit's ripening state, as Wood Mice avoid unripe green fruits (Authors, unpublished data) that are known to contain defensive compounds (Holliman & Milton 1990; Werner & Ebel 1994; Cooper 1998). We have explored the interaction between *H. foetidus* and the Wood Mouse to evaluate the effect of fruit ripening on mouse foraging and assessed whether such an effect holds constant across a micro-landscape that differs in foraging costs such as predation risk.

In this study, we used standardized food patches that simulated fruiting *H. foetidus* plants to establish fruit preferences of the Wood Mouse. We also examined the foraging of the Wood Mouse on *H. foetidus* fruits under contrasting perceived predation risks by means of a procedure that allowed us to isolate the effects of fruit ripening stage and predation risk on foraging by mice. The predominant defensive compound of *H. foetidus* (protoanemonine) acts as a digestibility reducer (Knight & Walter 2003). Foraging theory (Schmidt 2000) predicts that as the predation cost increases, the marginal value of foods containing digestibility reducers declines relative to that of the nontoxic foods. Thus, we expected that unripe *H. foetidus* fruits containing a higher amount of digestibility-reducing toxins would be relatively less depleted than would ripe fruits under higher predation risk. Specifically, our experimental approach aimed to answer the following four questions: (1) Does the ripening stage of *H. foetidus* fruits affect foraging by field mice? (2) If so, are the strength and direction of this effect consistent when fruit ripening varies at different hierarchical levels (e.g. between plants vs. within plant)? (3) Is the effect of ripening stage on mouse foraging consistent in strength and direction under differing risks of predation? and (4) What is the relative importance of fruit ripening vs. predation risk in determining foraging by frugivorous mice?

Methods

STUDY SITES AND SYSTEM

The study was carried out in the Cazorla Mountains of south-eastern Spain (37°56′ N, 2°52′ W) during the summers (May–June) of 2003 and 2004. To include a representative range of ecological conditions (mice abundance, food preferences, microhabitats) in which the *H. foetidus*–mice interaction takes place, we selected three plant populations within the Guadahornillos watershed (Roblehondo, Aguaderillos, Barranco de la Charca) at an elevation of 1250–1300 m. Populations were located within mixed forests of pine (*Pinus nigra*) and oak (*Quercus rotundifolia*) trees with variable understory of *Juniperus oxycedrus*, *Rubus ulmifolius* and *Daphne laureola*. The substrate was a mosaic of bare or grassy ground and highly fissured rock outcrops. Separation among target *H. foetidus* populations ranged between 0·7 and 2·1 km, ensuring that mice did not move between populations (Fedriani 2005). Climate was Mediterranean, characterized by dry, hot summers and relatively mild, wet winters. Average annual rainfall in the Cazorla Mountains ranges between 560 and 1660 mm.

Helleborus foetidus is an abundant perennial herb in the understory of mixed forests and scrublands of Cazorla. Plants typically consist of one to three reproductive ramets, each typically producing nine to 29 fruits that ripen early in the summer (May–June; Fedriani 2005). Flowers are apocarpous, usually with two to three carpels. Each carpel releases eight to 15 elaiosome-bearing seeds (Herrera *et al*. 2002). During their ontogenetic development, fruits show profound changes in size, colour and brightness. Four stages can clearly be distinguished (Fig. 1). Mice appear to select

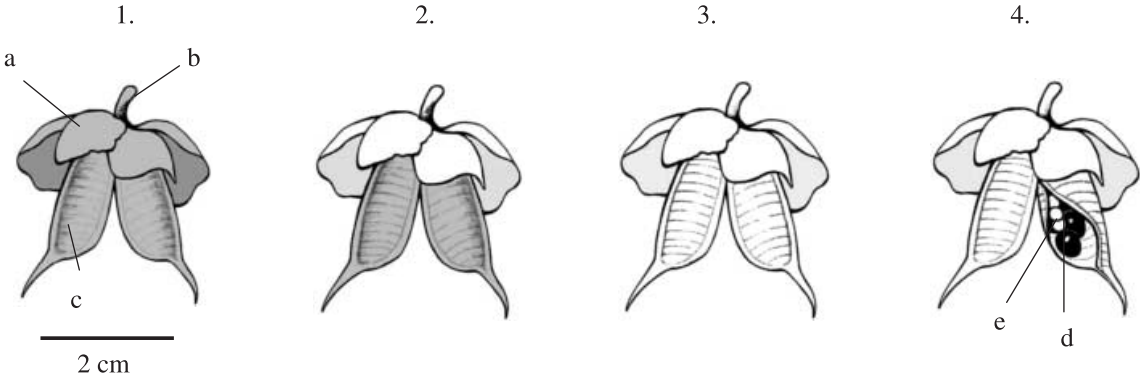

Fig. 1. Illustration of the ontogenetic development of *H. foetidus* fruits. (1) Unripe fruit: (a) sepals; (b) pedicel; and (c) carpels are all bright and intensively green in colour. (2) Mid-ripe fruit: carpels intensively green and the remaining structures are pale yellow. (3) Ripe fruit: carpels and adjacent structures are all pale yellow. (4) Dehiscing fruits: like ripe fruits that after 1–3 days split open length-wise, making their seeds (d) visible with their attached elaiosomes (e). The duration of the unripe fruits in *H. foetidus* lasts up to several months. The transition from mid-ripe to ripe fruits takes about 1 week, and the transition from ripe to dehiscing is 2–4 days. Dehiscing fruits release their seeds within 1–2 days. Mice mostly prey on mid-ripe and unripe fruits.

the mid-ripe fruits compared with unripe fruits, potentially due to unripe fruits containing higher levels of toxicity (Bonora, Dall'Olio & Bruni 1985; Holliman & Milton 1990; Werner & Ebel 1994; Cooper 1998; JM Fedriani unpublished data). Mice rarely feed on ripe and dehiscing fruits presumably because they are ephemeral. Lactone protoanemonine is the main toxin in *H. foetidus* (Bonora *et al*. 1985; Holliman & Milton 1990; Bai *et al*. 1996; Cooper 1998; Knight & Walter 2003), and its concentrations in the aerial parts of the plant can be as high as 672 $\mu g\ g^{-1}$ of wet weight (Bonora *et al*. 1985); however, the amount of protoanemonine in *H. foetidus* plants may show noticeable spatial, temporal and phenological (e.g. ripening stage) variation (J.M. Fedriani *unpublished data*). Protoanemonine lethal doses (LD_{50}) for laboratory mice *Mus musculus* weighing 20 g is $\approx 3{\cdot}8$ mg (Martin, San Roman & Dominguez 1990). *Helleborus foetidus* fruits do not show obvious physical defences against mice. No other rodent or even mammal species (including livestock) removes *H. foetidus* fruits at any stage of development in the Cazorla Mountains. Other details on the ecology of *H. foetidus* in the Iberian Peninsula can be found in Herrera *et al*. (2002), Fedriani *et al*. (2004) and Rey *et al*. (2006).

Wood Mice (*Apodemus sylvaticus*) are small rodents (14–28 g) common in mixed forests and scrublands of the Cazorla Mountains. Mice remove up to 52% of the *H. foetidus* fruits (Fedriani *et al*. 2004). Though it is not possible to know the fate of all seeds removed by mice (Hulme & Kollmann 2005; Vander Wall, Kuhn & Beck 2005), several lines of evidence indicate that mice act exclusively as seed predators (not dispersers) of *H. foetidus* (Fedriani *et al*. 2004). Based on previous knowledge of the system we anticipated three possible hierarchically nested levels in which ripening stage could affect mouse foraging. First, foraging mice must choose an individual plant among individuals available in the population based on whole plant ripening stage (i.e. 'among individuals selection'). Secondly, when the selected plant has more than one fruiting ramet (which occurs frequently), a mouse has to assess ramet ripening and select the one based on whole ramet ripening stage ('between ramets selection'). Thirdly, a mouse must climb the selected ramet to reach and assess the fruits (i.e. 'within ramet selection'). Mice remove fruits from the plant ramets, one at a time, by climbing the plant and chewing the fruit pedicels (Herrera *et al*. 2002). Once the selected fruits fall to the ground, mice carry them to safe microsites (i.e. rock crevices) where they extract the seeds by chewing and partially consuming the tender carpel walls (Fedriani & Manzaneda 2005; JM Fedriani personal observation). Therefore, Wood Mice belong to the 'fearful frugivores' category (*sensu* Howe 1979), which forage singly and remove few fruits and hide in nearby refuges to process them.

MOUSE MULTILEVEL RESPONSE TO FRUIT RIPENING

Mouse response to fruit ripening was evaluated with captive mice by quantifying their preference for unripe vs. mid-ripe *H. foetidus* fruits (Fig. 1). These two fruit stages were chosen because they are the ones usually depredated by mice. For convenience, however, we hereafter will refer to these two stages (unripe and mid-ripe) as unripe and ripe fruits, respectively. Mice were live-trapped in the three focal populations using Sherman traps baited with peanut butter during late May and early June of 2003. Forty to 60 traps per population were set during up to four consecutive nights in or very close to the sites where subsequent experiments were conducted (see below), and checked daily within 2 h after sunrise. Ten captured nursing females or young individuals (< 20 g) were immediately released. The remaining captured mice ($n = 17$) were housed individually in mesh cages ($100 \times 50 \times 50$ cm) that had

a piece of pipe (centred in the cage) with pressed cotton bedding where animals could hide during daytime and night breaks. Cages were placed in a quiet room under ambient temperature and photoperiod at our field station in the Cazorla Mountains. Two windows allowed some lunar light to enter, but the room was under dim light conditions during the fruit choice trials, thus resembling nocturnal luminance under field conditions (i.e. nocturnal luminance under tree canopy). The sides of the cages were covered to preclude mice from seeing each other. Mice were offered apples shortly after capture as well as after completion of each trial, but no food other than *H. foetidus* fruits was provided during trials (water was provided *ad libitum* at all times). To minimize the time mice were captive, fruit-choice trials started the first night after capture.

Fruiting *H. foetidus* plants differ greatly in attributes other than ripening stage, such as crop size, number of reproductive ramets, and fruit size (Guitián *et al*. 2003; Fedriani 2005; Herrera 2005). To control for the potential effects of these factors and thus isolate the effect of fruit ripening on foraging by mice, fruits were offered in standardized food patches or 'artificial plants' that differed in the ripening stage of offered fruits, but were otherwise virtually identical. Fruits were arranged in a way that resembled their presentation in natural plants (Fig. 2). Each artificial plant was composed of two plastic stems similar in size to reproductive ramets (50 cm height, ≈ 1 cm diameter) set vertically and separated by 30 cm. Two artificial plants were placed at the opposite side of each cage at about 90 cm from each other. Six pairs of fruits were fixed by their pedicels with transparent adhesive tape along each perch (overall, 24 fruits per artificial plant; Fig. 2). Adjacent pairs of fruits were separated by about 5 cm with the first pair of fruits always 10 cm from the ground (Fig. 2). Only two-carpel fruits without anomalous shape or size or signs of fungus infection or shrivelled appearance were used. Fruits were collected from a neighbouring population. In order to minimize potential differences (other than ripening stage) of offered fruits, collected fruits from different plants were pooled in two samples, one for unripe and one for ripe fruits, which were used to make the artificial plants.

During each trial, 24 unripe and 24 ripe *H. foetidus* fruits were offered according to three different modalities: (1) to assess the effect of fruit ripening stage on mouse foraging at the between-plants level, we ensured that one artificial plant had all 24 unripe fruits whereas the other had all ripe fruits; (2) to assess the effect of fruit ripening on mouse foraging at between-ramets level, one ramet of each plant had all 12 unripe fruits whereas the other had all ripe fruits; and (3) to assess the effect of fruit ripening on mouse foraging at the within-ramet level, each ramet of both plants contained six unripen fruits and six ripe fruits (in this case, each pair of fruits were composed by one unripe and one ripe fruit; Fig. 2). Trials started at 21.00 h and the numbers of unripe and ripe fruits removed by mice were recorded after 9 h. An effect of trial modality in the overall number of fruits removed would be indicative of contrasting foraging costs across trial modalities. As mice did not usually eat all the fruits they removed, we also recorded the number of fruits consumed. Mice were released at their respective capture sites after they were submitted in random order to each of the three trial modalities.

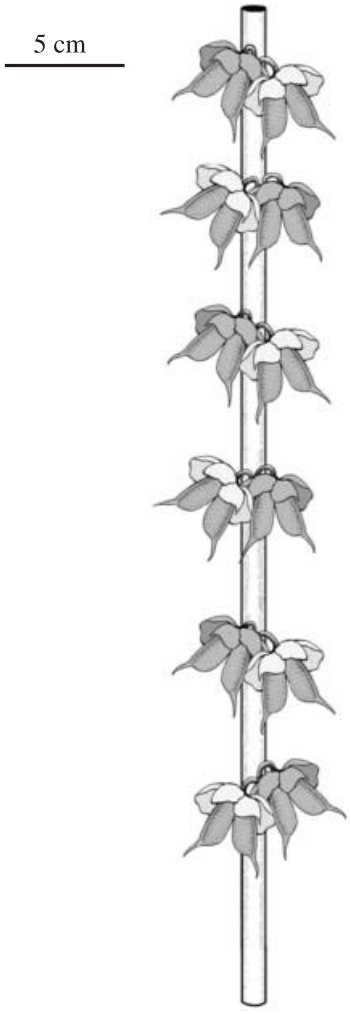

Fig. 2. Sketch of an artificial infructescence holding mid-ripe and unripe *H. foetidus* fruits as in our 'within ramet selection' trials with captive mice. The sketch also corresponds with the artificial infructescences used in our field experiments.

COMBINED EFFECT OF FRUIT RIPENING AND PREDATION RISK ON MOUSE FORAGING

To evaluate the effects of ripening stage of *H. foetidus* fruits and predation risk on mouse foraging, we carried out field experiments in each population during late June 2004 (immediately after seed release). Free-ranging mice were offered artificial plants similar to those used in trials with captive mice. Each artificial plant consisted of a unique ramet with 12 fruits set in six pairs (Fig. 2). Plants were affixed vertically to the ground surface. Fruits were collected from a neighbouring

population. Only healthy fruits of two carpels were used and fruits belonging to different plants were mixed together to minimize the potential effect of differences among plants in fruit traits. Within each pair of fruits, one was unripe and the other was ripe (Fig. 2); hence, we assessed the effect of fruit ripening on mouse foraging at the 'within-ramet' level described above. This level of selection was chosen because if free-ranging mice respond at this fine-grained level, we could assume they would respond at coarser levels (i.e. among plants, between ramets). To evaluate the role of predation risk on mouse foraging on *H. foetidus*, we considered rocky and bare substrates that were known to correspond with low- and high-perceived risks of predation for Wood Mice, respectively (Fedriani 2005). Artificial plants were set in pairs and, from each pair (or block), one plant was located in rocky substrate, whereas the other was separated by about 0·9 m and located in bare ground with little or no vegetation. In each population, we set 15 blocks (i.e. 30 artificial plants) and spacing among blocks was 3–5 m. Thus, there were a total of 90 artificial plants and 1080 fruits. Any remaining fruits were replaced daily to prevent desiccation of offered fruits as well as diminution of volatile defensive compounds (Cooper 1998; Knight & Walter 2003). The cumulative number of fruits of each type removed over three consecutive nights was recorded for each artificial plant.

STATISTICAL ANALYSES

To evaluate whether *H. foetidus* fruit ripening affected foraging by captive mice and whether such an effect was consistent across different trial modalities (testing effects at the between plants, between ramets, and within ramet levels), we fit a generalized linear mixed model with binomial error and logit link function using the SAS macro GLIMMIX (Littell *et al*. 1996); with the number of fruits removed per trial [divided by the number of fruits offered (n = 24 fruits of each type)] being the dependent variable. In this analysis, individual mice (nested within population) and population were considered as random factors (Bennington & Thayne 1994). As mice did not consume all fruits removed during each trial (see below), we further fit two comparable models in which the response variables were, first, the number of fruits consumed divided by the number of fruits offered and, second, the number of fruits consumed divided by the number of fruits removed. In preliminary analyses, mice 'gender' and all its possible second- and third-order interactions with 'trial modality' and 'fruit ripening' were also considered. However, 'gender' and its interactions had no effect on the dependent variables and therefore were not further considered. Model-adjusted means and standard errors were calculated and back-transformed using the appropriate Taylor's series approach (Littell *et al*. 1996).

To assess the combined effect of fruit ripening and predation risk on mouse foraging, we also fit a generalized linear mixed model with binomial error and logit link function using SAS macro GLIMMIX. The dependent variable was the cumulative number of fruits removed per artificial plant after 3 days of exposure to mice divided by the number of fruits offered (six fruits of each type per simulated plant). Pairs of artificial plants or blocks (nested within population) and population were included in the model as random factors. Fruit ripening stage and predation risk (low in rocky substrate vs. high in bare ground) and their second-order interaction were included in the model as fixed factors. As the interaction between fruit ripening stage and predation risk was significant (see below), we performed tests for the effect of a given factor tested at the different levels of the other factor ('tests of simple main effects') using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell *et al*. 1996). Model-adjusted means and standard errors were calculated and back-transformed using the appropriate Taylor's series approach.

Our methodology differs somewhat from other studies investigating the interaction between plant chemical defences and predation risk (Schmidt *et al*. 1998, 2000; Hochman & Kotler 2006), but the conceptual framework is essentially the same. Previous studies have adopted a 'patch-use approach' (Brown 1988) using food patches comprising food items (usually seeds) mixed within a matrix of sand. Such procedure ensures that foragers experience diminishing returns, and ecologists have used the amount of food remaining in the patch when a forager leaves (GUD) as response variable. In our study, simulated fruiting plants resembled natural conditions under which Wood Mice typically encounter *H. foetidus* fruits. This maximized realism of our experimentations but whether Wood Mice experienced diminishing returns depended of whether access to *H. foetidus* fruits became more difficult as fruit was removed. Therefore, we used number of fruits removed (or eaten) instead of GUDs as metric of consumption. Our procedure ensured that experimental units (i.e. simulated plants) yielded comparable measurements of consumption for each experiment and, thus, allowed for rigorous comparisons of the number of fruits removed or eaten by mice under different treatment combinations.

Results

MOUSE MULTILEVEL RESPONSE TO FRUIT RIPENING

Seventeen individuals (13 males and four females) were initially selected for the fruit selection trials (four, five and eight individuals from Barranco de la Charca, Aguaderillos and Roblehondo, respectively). These individuals appeared to behave normally, remaining inside the pipe during most of the day and being active at night. Video tape recordings on four individuals showed that captive mice climbed up the ramets, chewed the pedicels of the fruits to drop them and,

Table 1. Main results from generalized linear mixed models assessing the effect of *H. foetidus* fruit ripening stage on foraging by 15 captive mice. During each trial, unripe and ripe *H. foetidus* fruits (24 of each) were offered to individual mice on artificial plants made up of two ramets. Each mouse experienced three trial modalities in order to evaluate the effect of fruit ripening on mouse foraging at the 'between plants', 'between ramets', and 'within ramet' levels. We first modelled data on the number of fruits removed divided by the number of fruits offered per trial. As mice did not consume all fruits removed but often left some of them, we also modelled the number of fruits of each type consumed per trial divided by the number of fruits offered, and the number of fruits consumed divided by the number of fruits previously removed. Significant results ($P < 0{\cdot}05$) in **boldface**

	Fruits removed/ fruits offered			Fruits consumed/ fruits offered			Fruits consumed/ fruits removed		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Trial modality (TM)	2, 66	0·69	0·504	2, 66	0·51	0·604	2, 61	0·16	0·854
Ripening stage (RS)	**1, 66**	**10·97**	**0·002**	**1, 66**	**12·52**	**0·001**	1, 61	3·31	0·074
TM × RS	2, 66	0·02	0·984	2, 66	0·66	0·519	2, 61	0·49	0·613

once on the ground, chewed and partially consumed the tender walls of the carpels to extract the seeds. Nevertheless, two males from Roblehondo died during the first night due to unknown reasons. Therefore, data presented hereafter refer to 15 individuals, which were subjected to 43 feeding trials (all individuals but one experienced the three trial modalities described above).

Mice removed 32·4% of fruits offered during the fruit selection trials (685 of 2112 fruits). There were no significant effects of population of origin or individual on the number of fruits removed per trial ($z < 0{\cdot}68$, $P > 0{\cdot}284$). As expected, there was a strong effect of fruit ripening stage on mouse foraging (Table 1), with mice significantly avoiding unripe fruits (see 'Removed' in Fig. 3). This pattern was consistent at the three levels of mouse response tested (between plants, between ramets, within ramet) as indicated by the lack of significant effect of the interaction between trial modality and fruit ripening (Table 1). Specifically, corrected mean numbers of fruits removed by mice were 1·7–2·0-fold lower for unripe compared with ripe fruits (Fig. 3). Trial modality had no effect (Table 1), indicating that the overall number of fruits removed by mice was consistent at the three levels of response tested and suggesting similar foraging costs across trial modalities.

The overall percentage of fruits consumed by mice was 15·2% (321 of 2112 fruits). Neither population nor individual had a significant effect on the number of fruits consumed of those offered ($z < 0{\cdot}76$, $P > 0{\cdot}225$). Trial modality also had no effect on the number of fruits consumed (Table 1). As for fruit removal, fruit ripening stage affected fruit consumption by mice ($P < 0{\cdot}001$; Table 1), which was 1·5–2·8 times lower for unripe than for ripe fruits (see 'Eaten [I]' in Fig. 3). Therefore, results based on the number of fruits consumed by mice were generally consistent with those found for fruit removal (Fig. 3). However, mice consumed only 46·6% of the fruits previously removed from artificial plants (Fig. 3). Interestingly, consideration of the proportion of fruits consumed from those previously removed reveals that fruit ripening stage had only a marginal effect ($P = 0{\cdot}074$; Table 1), suggesting that, once fruits were on the ground, mice were less responsive to fruit ripening. This unexpected result is partly explained by the fact that the proportion of fruits consumed varied widely and significantly among individual mice ($z = 1{\cdot}92$, $P < 0{\cdot}028$), reflected by the large standard errors around the mean values of the proportion of fruits consumed (see 'Eaten [II]' in Fig. 3). Overall, mean percentages (±1 SE) of fruits consumed from those previously removed were 37·8 ± 46·1% and 51·9 ± 46·5% for unripe and ripe fruits, respectively. As above, trial modality and its interaction with fruit ripening did not affect the proportion of fruits consumed from those previously removed (Table 1).

COMBINED EFFECT OF FRUIT RIPENING AND PREDATION RISK ON MOUSE FORAGING

Fruit removal by free-ranging mice was quite variable, with some pairs of plants (blocks) not encountered (or ignored) and others heavily predated (block effect, $z = 3{\cdot}56$, $P < 0{\cdot}0002$). Less variation was found across populations ($z = 0{\cdot}27$, $P = 0{\cdot}394$). After controlling for the effect of random factors, both fruit ripening stage ($F_{1,132} = 19{\cdot}6$, $P < 0{\cdot}0001$) and predation risk ($F_{1,132} = 111{\cdot}6$, $P < 0{\cdot}0001$) had strong effects on mouse foraging. However, the interpretation of their effects is not straightforward because of their joint effect (Fruit ripening–Predation risk interaction, $F_{1,132} = 14{\cdot}58$, $P < 0{\cdot}0002$; Fig. 4A). Tests of simple main effects indicated that fruit ripening influenced the number of fruits removed from artificial plants located in microhabitats with low predation risk (rocky substrates; $F_{1,132} = 94{\cdot}80$, $P < 0{\cdot}0001$), but not from those located in microhabitats with high predation risk (bare ground; $F_{1,132} = 0{\cdot}12$, $P = 0{\cdot}734$; Fig. 4A). At low perceived predation risk, ripe fruits were removed 7·5 times more often than unripe fruits (Fig. 4A). No effect of fruit ripening on mouse foraging was found at high-perceived predation risk, as mice removed few fruit. Not surprisingly, tests of simple main effects also indicated that predation risk affected the number of unripe ($F_{1,132} = 26{\cdot}91$, $P < 0{\cdot}0001$) and ripe fruits removed ($F_{1,132} = 116{\cdot}05$, $P < 0{\cdot}0001$; Fig. 4A). Because a non-negligible fraction (38%, $n = 45$) of experimen-

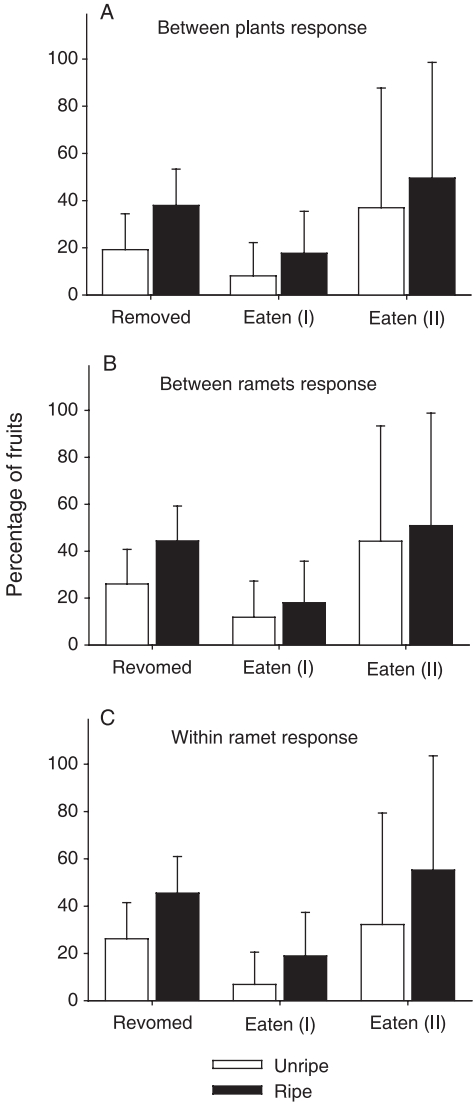

Fig. 3. Model-adjusted means (± 1 SE) of percentages of *H. foetidus* fruits of each type (unripe and ripe) removed by captive mice considering first the number of fruits offered per trial (n = 24 fruits of each type). As mice did not consume all fruits removed during each trial, we further fitted two comparable models in which the response variables were, first, the number of fruits consumed divided by the number of fruits offered (Eaten [I]) and secondly, the number of fruits consumed divided by the number of fruits previously removed (Eaten [II]). Each mouse experienced three trial modalities testing the effect of fruit ripening on mouse foraging at the 'between plants' (A), 'between ramets' (B), and 'within ramet' levels (C).

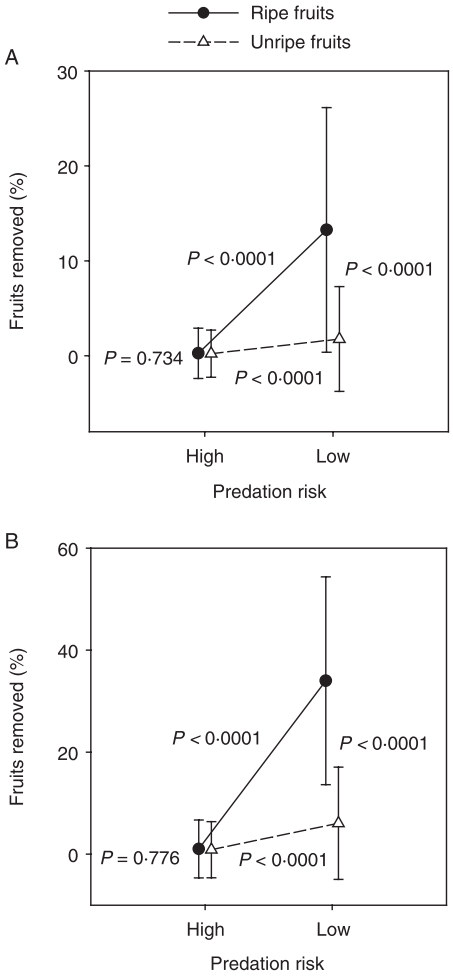

Fig. 4. Model-adjusted means (± 1 SE) of the percentages of number of *H. foetidus* fruits removed by free-ranging mice during three consecutive nights from artificial plants holding unripe and ripe fruits. Plants were located either at high or low perceived risk of predation for mice (i.e. bare grounds and rocky substrates, respectively). In the first analysis we considered all experimental blocks (A) and, in the second analysis, only those blocks encountered by mice (i.e. at least one fruit removed by mice; B). In both analyses the interaction between fruit ripening stage and predation risk was significant. Therefore, we report the P-values of the tests for the four simple main effects involved in the interaction.

tal blocks were nonencountered or ignored by mice (i.e. no fruit was removed), we performed a second analysis considering only those blocks where at least one fruit was removed. Fruit ripening stage ($F_{1,79}$ = 13·91, P < 0·001) and predation risk ($F_{1,79}$ = 81·29, P < 0·0001) as well as their interaction ($F_{1,79}$ = 10·33, P = 0·002; Fig. 4B) continued to have strong effects on the number of fruits removed. Tests of simple main effects also indicated that fruit ripening influenced the number of fruits removed in rocky substrates ($F_{1,79}$ = 64·42, P < 0·0001), but not from those located in bare ground ($F_{1,79}$ = 0·08, P = 0·776; Fig. 4B). Besides, we found that predation risk affected the number of both unripe ($F_{1,79}$ = 18·63, P < 0·0001) and ripe fruits removed ($F_{1,79}$ = 83·91, P < 0·0001; Fig. 4B). These results corroborate that the effect of fruit ripening on mouse foraging took place only in plants located in microhabitat with low perceived predation risk (rocky substrate) but not in microhabitats with high predation risk (bare ground).

Discussion

Mice preferred ripe over unripe fruit at all spatial scales. Mice not only avoided artificial plants with unripe fruit crops, they also responded to within-crop ripening, avoiding unripe ramets within plants and

unripe fruits within ramets. These results are consistent with the idea that unripe fruits of many plants have a variety of secondary compounds (i.e. toxins, digestion inhibitors) that are gradually reduced during ripening (Cipollini & Levey 1997; Herrera 2002; Izhaki 2002; Schaefer *et al*. 2003). Previous research, including our own preliminary chemical analyses (J.M. Fedriani unpublished data), suggests that this is also the case for *H. foetidus* fruits. For example, when chewed, *H. foetidus* rapidly releases an irritating oil, protoanemonine, that acts as a digestive inhibitor, resulting in irritation to the mucous membranes of the mouth and digestive system (Holliman & Milton 1990; Bai *et al*. 1996; Cooper 1998; Knight & Walter 2003). Protoanemonine, the main toxin of Ranunculaceae, is a volatile lactone that, upon drying, polymerizes to the nontoxic anemonine (Holliman & Milton 1990; Bai *et al*. 1996; Jurgens & Dotterl 2004). Though the nutritive value of *H. foetidus* fruits may also vary during ripening, amounts of protoanemonine in dry ripe *H. foetidus* fruits is lower than in turgid unripe ones and presumably accounts for the avoidance of unripe fruit by mice in our study.

The effect of plant secondary compounds on herbivore foraging decisions depends on the physiological mechanisms by which these compounds render food undesirable (Schmidt *et al*. 1998; Schmidt 2000). Foraging theory (Schmidt 2000) predicts that, all else being equal, foragers under higher predation risk will harvest fewer fruit that contain digestibility-reducing toxins than fruit without such defences because, as predation costs increase, the marginal value of food containing digestive reducers declines relative to that of nontoxic foods. Interestingly, however, results from our field experiments did not support this prediction. In the safe microhabitat (rocky substrate) unripe fruits containing higher amounts of protoanemonine were, as expected, avoided compared with ripe fruits (Fig. 4). However, in the risky microhabitat (bare ground) there was no effect of ripening stage on fruit consumption by mice. Several possibilities can explain this unexpected result. First, as found for some desert rodents (e.g. *Gerbillus pyramidum*, *G. allenbyi*; Brown, Kotler & Valone 1994), predation risk appeared to dominate the costs of wood mice foraging on *H. foetidus* fruits. Consequently, the number of fruits removed of either ripening stage in the risky microhabitat (bared grounds) was too low to detect an effect of fruit ripening. As a forager's attention is increasingly directed towards predators, the less it is directed towards foraging tasks (Schmidt & Brown 1996; Fierer & Kotler 2000; Kotler, Brown & Bouskila 2004), mice under a high risk of predation might be less able to distinguish between fruits of differing ripeness. Secondly, protoanemonine may show a dose-dependent effect and simulated plants could have been encountered by different individual mice (or in different nights by the same individual). If so, in the risky microhabitat, where mice consumed too few fruits, a low dose of protoanemonine may have not produced an effect on mice foraging and, thus, ripe and unripe fruit should be of near equal value. In safe environments, where mice consumed more fruits of both kinds, protoanemonine should have a stronger effect on mice foraging and fewer unripe fruits were consumed. Finally, other chemical defences less abundant in *H. foetidus* (e.g. cardiac glycosides; Holliman & Milton 1990) might act as toxins rather than as digestibility reducers. If so, the combined effect of such diverse defensive compounds on mice fruit choice may depart from expectations based on the presence of a single compound type (Schmidt *et al*. 1998; Schmidt 2000).

As mentioned above, fruit ripening affected foraging by captive mice as they harvested fewer unripe than ripe fruits. Surprisingly, however, mice appeared less sensitive to fruit ripening once fruits were on the ground (Fig. 3). We propose that mice might have used a simple mechanism for 'handling' *H. foetidus* toxins that enabled them to consume some unripe fruits (see Dearing, Foley & McLean 2005 for review). Indeed, intensive monitoring of fruiting *H. foetidus* in the three focal populations during 2003 and 2004 (overall, 180 plants) indicated that removed unripe *H. foetidus* fruits are not always consumed immediately, but are in fact 'stored' beneath the parent plants during 1–3 days before consumption (authors' unpublished data). Moreover, limited data from videotape records of trials of four captive mice indicated that removed fruits were usually not eaten until several hours after being removed. Thus, once unripe fruits are on the ground, it is likely that wood mice perceived *H. foetidus* defensive compounds (by smell, squeezing, or even testing the carpels) and delayed consuming fruits until the concentration of defensive compounds decreased below a tolerable level (e.g. Dearing 1997).

The consistence in the pattern of fruit choice by captive mice across different levels (i.e. between plants, within plant) suggests mice have the ability to exert phenotypic selection on plant traits such as fruiting phenology and synchrony, and plant defensive compounds (Cipollini & Levey 1997; Herrera 2002). It is interesting to note the prolonged life of unripe *H. foetidus* fruits in contrast with the ephemeral nature of their ripe fruits (Fig. 1). Conceivably, as pre-dispersal seed predation by mice tends to occur on ripe fruits, mice may be acting as agents of selection for a rapid seed drop by ripe *H. foetidus* fruits, which would minimize seed exposure to mice. However, as reported for other plant–frugivore systems (Sallabanks 1993; Jordano 1995), fruit predation by mice appeared to be a multistep process that responded to the environment (substrate) in which *H. foetidus* plants were located. As the percentage of plants located in rocky substrates can vary among populations as much as 0–67% (Fedriani 2005), we propose that selection pressure by mice on *H. foetidus* can be limited to populations dominated by rocky substrates. Thus, the strength of selection by mice on *H. foetidus* is likely to vary across their space of coexistence due to the effect of both biotic (i.e. mouse

abundance) and abiotic factors (i.e. substrate type), which is consistent with recent theories on the geographic structure of interactions (Thompson 1994, 2005).

To ecologists, factors such as abundance and distribution of resources and predators are paramount determinants of foraging, while for physiologists foraging is most often determined by factors such as energetic value and proportion of digestive vs. refractory components of foods (Karasov & Diamond 1988; Whelan *et al*. 2000; Whelan & Brown 2005). Frugivorous mice responded to predation risk as well as to chemical defensive compounds present in unripe *H. foetidus* fruits. Such responses, however, were sequential and only when mice selected microhabitats with low predation cost did plant defensive compounds appear to affect mouse foraging. This study provides novel empirical support for the necessity to consider both digestive (secondary compounds) and ecological (microhabitat, predation risk) factors to fully understand animal foraging, as well as selection pressures exerted by herbivores on plant populations.

Acknowledgements

We thank Jose A. Dorante, David Andrew, and Ibama Pineda for their enthusiastic field assistance. We thank Chris Whelan, Ken Schmidt, Victor Parra-Tabla, Carlos M. Herrera, Javier Guitian, and Craig W. Benkman for numerous constructive comments and editorial suggestions on early drafts. Natasha Wiggins and Hala Abdallah kindly reviewed our English. We thank Juaquín Lopez-Rojas for drawing the fruits and the artificial infrutescence. Consejería de Medio Ambiente (Junta de Andalucía) provided working facilities. We thank Ministerio de Educación y Ciencia for CGL2004-00094/BOS and BOS2000-1122-C03-02 grants.

References

Bai, Y., Benn, M.H., Majak, W. & McDiarmid, R. (1996) Extraction and HPLC determination of ranunculin in species of the buttercup family. *Journal of Agriculture Food Chemistry* **44**, 2235–2238.

Bennington, C.C. & Thayne, W.V. (1994) Use and misuse of mixed model analysis of variance in ecological studies. *Ecology* **75**, 717–722.

Bonora, A., Dall'Olio, G. & Bruni, A. (1985) Separation and quantitiation of protoanemonin in Ranunculaceae by normal- and reversed-phase HPLC. *Planta Medica* **51**, 364–367.

Bozinovic, F. & Martínez del Río, C. (1996) Animals eat what they should not: why do they reject our foraging models? *Revista Chilena de Historia Natural* **69**, 15–20.

Brown, J.S. (1988) Patch use as an indicator of habitat preference, predation risk, and competition. *Behavioral Ecology and Sociobiology* **22**, 37–47.

Brown, J.S. & Kotler, B.P. (2004) Hazardous duty pay and the foraging cost of predation. *Ecology Letters* **7**, 999–1014.

Brown, J.S. & Mitchell, W.A. (1989) Diet selection on depletable resources. *Oikos* **54**, 33–43.

Brown, J.S., Morgan, R.A. & Dow, B.D. (1992) Patch use under predation risk: II. A test with fox squirrels, *Sciurus niger*. *Annales Zoologica Fennici* **29**, 311–318.

Brown, J.S., Kotler, B.P. & Valone, T.J. (1994) Foraging under predation – a comparison of energetic and predation costs in rodent communities of the Negev and Sonoran deserts. *Australian Journal of Zoology* **42**, 435–448.

Cipollini, M.L. & Levey, D.D.L. (1997) Secondary metabolites of fleshy vertebrate-dispersed fruits-adaptative hypotheses and implication for seed dispersal. *American Naturalist* **150**, 346–372.

Cooper, M.R. (1998) *Poisonous Plants and Fungi in Britain: Animals and Human Poisoning*. Her Majesty's Stationary Office, London.

Dearing, M.D. (1997) The manipulation of plant secondary compounds by a food hoarding herbivore, the North American pika, *Ochotona princeps*. *Ecology* **78**, 774–781.

Dearing, M.D., Foley, W.J. & McLean, S. (2005) The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annual Review of Ecology, Evolution and Systematic* **36**, 169–189.

Dudley, R. (2004) Ethanol, fruit ripening, and the historical origins of human alcoholism in primate frugivory. *Integrative and Comparative Biology* **44**, 315–323.

Fedriani, J.M. (2005) Do frugivorous mice choose where or what to feed on? *Journal of Mammalogy* **86**, 576–586.

Fedriani, J.M. & Manzaneda, A.J. (2005) Pre- and post-dispersal seed predation by rodents: balance of food and safety. *Behavioral Ecology* **16**, 1018–1024.

Fedriani, J.M., Rey, P., Garrido, J.L., Guitián, J., Herrera, C.M., Medrano, M., Sánchez-Lafuente, A. & Cerdá, X. (2004) Geographical variation in the potential of mice to constrain an ant-seed dispersal mutualism. *Oikos* **105**, 181–191.

Fierer, N. & Kotler, B.P. (2000) Evidence for micropatch partitioning and effects of boundaries on patch use in two species of gerbils. *Functional Ecology* **14**, 176–182.

Guitián, J., Medrano, M., Herrera, C.M. & Sánchez-Lafuente, A.M. (2003) Variation in structural gender in the hermaphrodite *Helleborus foetidus* (Ranunculaceae): within- and among-population patterns. *Plant Systematics and Evolution* **241**, 139–151.

Herrera, C.M. (2002) Seed dispersal by vertebrates. *Plant–Animal Interactions: an Evolutionary Perspective* (eds C.M. Herrera & O. Pellmyr), pp. 185–208. Blackwell Science, Oxford.

Herrera, C.M. (2005) Post-floral perianth functionality: Contribution of persistent sepals to seed development in *Helleborus foetidus* (Ranunculaceae). *American Journal of Botany* **92**, 1486–1491.

Herrera, C.M., Medrano, M., Rey, P.J., Sánchez-Lafuente, A., Gárcia, M.B., Guitián, J. & Manzaneda, A.J. (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Science USA* **99**, 16823–16828.

Hochman, V. & Kotler, B.P. (2006) Effects of food quality, diet preference and water on patch use by Nubian ibex. *Oikos* **112**, 547–554.

Holliman, A. & Milton, D. (1990) *Helleborus foetidus* poisoning of cattle. *Veterinary Record* **127**, 339–340.

Howe, H.F. (1979) Fear and frugivory. *American Naturalist* **114**, 925–931.

Hulme, P.E. & Kollmann, J. (2005) Seed predator guilds, spatial variation in post-dispersal seed predation and potential effects on plant demography: a temperate perspective. *Seed Fate: Predation and Secondary Dispersal* (eds P.M. Forget, J.E. Lambert, P.E. Hulme & S.B. Vander Wall), pp. 9–30. CAB International, Wallingford, Oxon.

Izhaki, I. (2002) The role of fruit traits in determining fruit removal in east Mediterranean ecosystems. *Seed Dispersal and Frugivory: Ecology, Evolution and Conservation* (eds D.J. Levey, W.R. Silva & M. Galetti), pp. 161–175. CAB International, Wallingford, Oxon.

Jordano, P. (1995) Frugivore mediated selection on fruit and

seed size: birds and St. Lucie's cherry, *Prunus mahaleb*. *Ecology* **76**, 2627–2639.

Jurgens, A. & Dotterl, S. (2004) Chemical composition of anther volatiles in ranunculaceae: Genera-specific profiles in *Anemone*, *Aquilegia*, *Caltha*, *Pulsatilla*, *Ranunculus*, and *Trollius* species. *American Journal of Botany* **91**, 1969–1980.

Karasov, W.H. & Diamond, J.M. (1988) Interplay between physiology and ecology in ingestion. *Bioscience* **38**, 602–611.

Knight, A.P. & Walter, R.G. (2003) Plants affecting the digestive system. *A Guide to Plant Poisoning of Animals in North America* (eds A.P. Knight & R.G. Walter). Teton NewMedia, Jackson WY.

Kotler, B.P., Brown, J.S., Oldfield, A., Thorson, J. & Cohen, D. (2001) Effect of escape substrate and foraging substrate on patch use by three species of gerbils. *Ecology* **82**, 1781–1790.

Kotler, B.P., Brown, J.S. & Bouskila, A. (2004) Apprehension and time allocation in gerbils: The effects of predatory risk and energetic state. *Ecology* **85**, 917–922.

Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. (1996) *SAS System for Mixed Models*. SAS Institute, Cary, NC.

Martin, M.L., San Roman, L. & Dominguez, A. (1990) In vitro activity of protoanemonin, an antifungal agent. *Planta Medica* **56**, 66–69.

Morgan, R.A., Brown, J.S. & Thorson, J.M. (1997) The effect of spatial scale on the functional response of fox squirrels. *Ecology* **78**, 1087–1097.

Morris, D.W. (1992) Scales and costs of habitat selection in heterogeneous landscapes. *Evolutionary Ecology* **6**, 412–432.

Rey, P.J., Herrera, C.M., Guitián, J., Cerdá, X., Sanchez-Lafuente, A.M., Medrano, M. & Garrido, J.L. (2006) The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology* **19**, 21–34.

Sallabanks, R. (1993) Hierarchical mechanisms of fruit selection by an avian frugivore. *Ecology* **74**, 1326–1336.

Schaefer, H.M., Schmidt, V. & Winkler, H. (2003) Testing the defence trade-off hypothesis: how contents of nutrients and secondary compounds affect fruit removal. *Oikos* **102**, 318–328.

Schaefer, H.M., Schmidt, V. & Levey, D.J. (2004) How plant–animal interactions signal new insights in communication. *Trends in Ecology and Evolution* **19**, 577–584.

Schmidt, K.A. (2000) Interactions between food chemistry and predation risk in fox squirrels. *Ecology* **81**, 2077–2085.

Schmidt, K.A. & Brown, J.S. (1996) Patch assessment in fox squirrels: the role of resource density, patch size, and patch boundaries. *American Naturalist* **147**, 360–380.

Schmidt, K.A., Brown, J.S. & Morgan, R.A. (1998) Plant defenses as complementary resources: a test with squirrels. *Oikos* **81**, 130–142.

Sumner, P. & Mollon, J.D. (2000) Chromaticity as a signal of ripeness in fruits taken by primates. *Journal of Experimental Biology* **203**, 1987–2000.

Thompson, J.N. (1994) *The Coevolutionary Process*. University of Chicago Press, Chicago, IL.

Thompson, J.N. (2005) *The Geographic Mosaic of Coevolution*. University of Chicago Press, Chicago, IL.

Vander Wall, S.B., Kuhn, K.M. & Beck, M.J. (2005) Seed removal, seed predation, and secondary dispersal. *Ecology* **86**, 801–806.

Werner, K. & Ebel, F. (1994) Zur Lebensgeschichte der Gattung Helleborus L. (Ranunculaceae). *Flora* **189**, 97–130.

Whelan, C.J. & Brown, J.S. (2005) Optimal foraging and gut constrains: reconciling two schools of thought. *Oikos* **110**, 481–496.

Whelan, C.J., Schmidt, K.A., Steele, B.B., Quinn, W.J. & Dilger, S. (1998) Are bird-consumed fruits complementary resources? *Oikos* **83**, 195–205.

Whelan, C.J., Brown, J.S., Schmidt, K.A., Steele, B.B. & Willson, M.F. (2000) Linking consumer-resource theory with digestive physiology: application to diet shifts. *Evolutionary Ecology Research* **2**, 911–934.

Received 6 June 2006; revised 3 August 2006; accepted 6 August 2006
Editor: Craig Benkman