RESEARCH ARTICLE

Physiological and behavioural effects of fruit toxins on seed-predating *versus* seed-dispersing congeneric rodents

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SUMMARY

Fleshy, ripe fruits attract seed dispersers but also seed predators. Although many fruit consumers (legitimate seed dispersers as well as seed predators) are clearly exposed to plant secondary compounds (PSCs), their impact on the consumers' physiology and foraging behaviour has been largely overlooked. Here, we document the divergent behavioural and physiological responses to fruit consumption of three congeneric rodent species in the Middle East, representing both seed dispersers and seed predators. The fruit pulp of the desert plant *Ochradenus baccatus* contains high concentrations of glucosinolates (GLSs). These GLSs are hydrolyzed into active toxic compounds upon contact with the myrosinase enzyme released from seeds crushed during fruit consumption. *Acomys russatus* and *A. cahirinus* share a desert habitat. *Acomys russatus* acts as an *O. baccatus* seed predator, and *A. cahirinus* circumvents the activation of the GLSs by orally expelling vital seeds. We found that between the three species examined, *A. russatus* was physiologically most tolerant to whole fruit consumption and even *A. minous*, which is evolutionarily naïve to *O. baccatus*, exhibits greater tolerance to whole fruit consumption than *A. cahirinus.* However, like *A. cahirinus*, *A. minous* may also behaviourally avoid the activation of the GLSs by making a hole in the pulp and consuming only the seeds. Our findings demonstrate that seed predators have a higher physiological tolerance than seed dispersers when consuming fruits containing toxic PSCs. The findings also demonstrate the extreme ecological/evolutionary lability of this plant–animal symbiosis to shift from predation to mutualism and *vice versa*.

Key words: *Acomys*, alanine aminotransferase, bitter taste, body mass, fruits, glucosinolates, liver, pulp, secondary compounds, seeds.

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INTRODUCTION

Fleshy, ripe fruit pulp is a nutritious and easily digestible reward that facilitates seed dispersal by animals (Herrera, 1982; Corlett and Lucas, 1990; Jordano, 2000; Izhaki, 2002a). However, fruits are likely to attract not only seed dispersers but also seed predators because seeds are also a valuable food source (Corlett and Lucas, 1990; Kelt et al., 2004). Ultimately, the nature of the plant–animal interaction is determined by the consumers' fruit eating strategy (Corlett and Lucas, 1990; Jordano, 2000; Dominy and Duncan, 2005).

Variability in fruit eating can be a function of multiple parameters, such as age, satiation/hunger, feeding location, seasonality, or availability of other food or water (Levey, 1987; Corlett and Lucas, 1990; Gautier-Hion et al., 1993; Kaplin and Moermond, 1998; Lambert, 1999; Samuni-Blank et al., 2012). In addition, fruits may contain plant secondary compounds (PSCs) to deter seed predators or alter the behaviour of the consumer (Cipollini and Levey, 1997; Samuni-Blank et al., 2012). In folivorous herbivores, evolutionary pressure has resulted in various behavioural and physiological adaptations for coping with PSCs, thus altering plant–herbivore interactions (McArthur et al., 1991; Dearing et al., 2005; Torregrossa et al., 2011). Similar adaptations to fruit PSCs may also influence fruit eating strategies and thus affect plant–animal interactions.

Glucosinolates (GLSs) are a common class of fruit PSCs. Intact GLSs are generally nontoxic and have a limited effect in defending the plant against herbivores and pathogens. The defensive properties of GLSs are enhanced upon their hydrolysis by the myrosinase enzyme, causing the release of a toxic aglycone (Hopkins et al., 2009). These unstable molecules rearrange into several toxic biological compounds (Kjaer, 1976; Das et al., 2000; Wittstock and Halkier, 2002). Generally, GLSs and myrosinases are compartmentalized to prevent toxicity to the plant, and only mix upon damage to tissue (Rask et al., 2000; Hopkins et al., 2009). The myrosinase–GLS system, also known as the mustard oil bomb, provides plants with an effective defense against generalist herbivores (Wittstock and Halkier, 2002).

The myrosinase–GLS defense system is employed in the fleshy fruit of *Ochradenus baccatus* Delile (Resedaceae), a common inhabitant of wadis and depressions in Israeli desert regions (Bronstein et al., 2007). The fleshy fruits of *O. baccatus* attract a number of consumers because of their high water and sugar content (Bronstein et al., 2007; Spiegel and Nathan, 2007; Spiegel and Nathan, 2011). However, the seeds of *O. baccatus* also represent a valuable nutrient source, as they are rich in protein (~25% of dry mass). To prevent seed predation, the fruits of *O. baccatus* defend

themselves with a unique arrangement of the mustard oil bomb, where GLSs are found in the pulp and the myrosinase enzyme is found in the seeds (Samuni-Blank et al., 2012). Thus, activation of the mustard oil bomb only occurs if consumers crush both seeds and pulp together.

Ochradenus baccatus naturally co-occurs with two congeneric rodent species [*Acomys russatus* (Wagner 1840) and *A. cahirinus* (Geoffrey 1803)] that differ in their interactions with the plant. *Acomys russatus* usually consumes the *O. baccatus* fruit as a whole, acting as a seed predator, while *A. cahirinus* consumes only the pulp and spits the seeds, thus acting as a seed disperser (Samuni-Blank et al., 2012; Samuni-Blank et al., 2013). These interactions result in differential exposure to activated GLSs between species: *A. russatus* consumes activated GLSs by masticating the seeds, while *A. cahirinus* consumes unactivated GLSs by expelling the seeds. We hypothesize that the variation in physiological adaptations to fruit PSCs drive these plant–animal interactions or *vice versa*. We also predict that toxin avoiders will show low physiological tolerance to whole fruit consumption and that the frequency of toxin avoiders within a species will be indicative of its tolerance.

The aim of the present study was to test the effects of fruits PSCs (pulp and seeds) on fruit consumers by preventing behavioural circumvention, while testing the physiological impacts of fruit PSCs on seed dispersers *versus* seed predators. For comparative purposes, we also documented the fruit-eating behaviour and physiological responses to fruit PSCs of the Crete spiny mouse [*A. minous* (Bate 1906)], which is endemic to the Mediterranean island of Crete and evolutionarily naïve to *O. baccatus* fruits. We presented each species with diets containing intact or activated GLSs, and monitored various physiological parameters such as body mass, food intake, dry matter digestibility and serum markers of liver damage. We predicted that the seed predator, *A. russatus*, would be physiologically more adapted to consume activated toxins, as it regularly does so in the wild. We also predicted that consumption of food containing activated GLSs would result in liver damage.

MATERIALS AND METHODS
Fruit collection
Fruits were collected from the Almog junction site (31°48′N, 35°27′E) located near the Dead Sea and kept at –20°C. For experiments in which separation of pulp and seeds was needed, fruits were kept at 4°C and pulp was manually separated from the seeds within 2 days of collection and then kept separately at –20°C. Stored fruits maintained active myrosinase and GLS content (Samuni-Blank et al., 2012). *Ochradenus baccatus* fruiting occurred year-round, with high peaks in May and November 2008–2012. Whole fruits and fruit pulp were thawed before use.

Animals and maintenance
The experimental protocols were approved by the University of Haifa Committee of Animal Experimentation (permit number 096/08). *Acomys cahirinus* [but see Barome et al. and Volobouev et al. (Barome et al., 2001; Volobouev et al., 2007) for a discussion on the *A. cahirinus*–*dimidiatus* complex) and *A. russatus* were from captive breeding colonies (at least three generations in captivity) originally established from individuals trapped in the vicinity of the Dead Sea (31°28′N, 35°23′E). Animals were maintained at the Department of Biology and Environment at the University of Haifa, Oranim. Adult individuals of *A. minous* were live-trapped in the vicinity of Heraklion, Crete, a few days prior to the experiment using Sherman folding traps placed under rocks. All captured *A. minous* individuals were maintained at the Natural History Museum of Crete

(Ministry of Environment, Energy and Climate Change, permit number 117272/586).

Prior to experiments, animals were fed rodent chow (Koffolk serial no. 19510, Tel Aviv, Israel) and fresh carrots as a source of free water, *ad libitum*. During the experiments, animals were housed individually in standard mouse cages (21×31×13 cm) in a temperature-controlled room (25±2°C) under a 12 h:12 h light:dark cycle. All animals were naïve to *O. baccatus* prior to the experiment.

Fruit-eating behaviour of *A. minous*
To determine the fruit-eating behaviour of *A. minous*, we placed each single animal (*N*=16) in a cage with five fruits overnight. The cage floor was examined the next morning for intact fruit parts (pulp or seeds) and the fruit-eating behaviour was classified as one of three types: (1) 'whole fruit' – eating pulp and seeds simultaneously and leaving no remains; (2) 'pulp' – eating the pulp and leaving the seeds; and (3) 'seed' – eating the seeds and leaving the pulp. Individuals that exhibited more than one fruit-eating behaviour were classified according to their dominant strategy. For example, if one individual ate two whole fruits and only the seeds of the remaining three fruits, then the behaviour was classified as 'seed'. We define avoiders as individuals that consume either pulp or seeds, but not both. We define confronters as individuals that consume the whole fruit, pulp and seeds simultaneously. We recorded the number of intact seeds dropped to the cage floor as well as the number of intact defecated seeds (in the feces). Similar experiments were previously performed on the two other *Acomys* species (Samuni-Blank et al., 2013).

Physiological effects of fruit toxins
To examine the physiological effects of different fruit eating strategies on the three rodent species, we performed controlled feeding trials. Diets included rodent chow (Koffolk serial no. 19510) mixed with homogenized pulp (pulp) or pulp and crushed seeds (mash; Table 1). The fruit pulp was free of myrosinase and contained only intact GLSs. The mash diet combined the GLSs from the pulp and the myrosinase enzyme from the seeds and therefore contained activated GLSs. To allow the animals to acclimate to the fruit diet, they were fed a 25% fruit diet (pulp or mash; wet weight) for 1 day combined with 75% chow, followed by 50% fruit mixed with 50% chow for three additional days. Wet food pellets (~50% water content) were prepared from the mashed food and given to the rodents within an hour of preparation (*N*=6–8 individual rodents per treatment).

Each experiment lasted 4 days. During the experiment, body mass and food intake were measured daily. Excreta and food leftovers were collected every day from the plastic cage floor, dried (50°C for 24 h) and weighed. Dry matter (DM) digestibility of food consumed by an animal was calculated from its DM food intake and fecal DM output as: DM digestibility=(DM food intake–fecal DM output)/DM food intake.

The effect of activated GLSs on the liver was studied through measurements of liver enzymes and liver function test. On the last

Table 1. Diet components (%)

		Mash	Pulp
Day 1	Chow	50	50
	Water	25	25
	Pulp	23	25
	Seeds	2	0
Days 2–4	Chow	50	50
	Water	0	0
	Pulp	46	50
	Seeds	4	0

Table 2. Summary of the fruit-eating behaviour of the three congeneric species of *Acomys*

	N	Pulp (%)	Seed (%)	Whole (%)	Reference
A. cahirinus	43	76.7	4.6	18.6	Samuni-Blank et al., 2013
A. minous	11*	9.1	54.5	36.4	Present study
A. russatus	43	11.6	0	88.4	Samuni-Blank et al., 2013

*Five other individuals did not consume any part of the fruit and were excluded.

day of the experiment, animals were euthanized with CO_2 and immediately dissected. Blood samples were collected by heart puncture and centrifuged (15 min at 600 *g*). Blood serum was collected and stored at –20°C. Samples were analyzed for alkaline phosphatase (ALP) and alanine aminotransferase (ALT). ALT is an intracellular enzyme of hepatocytes and its appearance in blood is indicative of possible liver damage. *Acomys cahirinus* and *A. russatus* samples were also analyzed for albumin, gamma-glutamyl transpeptidase (GGT), total bilirubin and direct bilirubin. Serum levels of all these enzymes and proteins are known to increase when hepatobiliary damage occurs (Ozer et al., 2008). All samples were analyzed at the Laboratory of Clinical Biochemistry in Rambam Medical Center, Haifa.

Statistical analyses
To test for differences between the fruit-eating behaviour of *A. minous*, we used a one proportion *Z*-test. Physiological responses on the last day of the diets (body mass, DM digestibility, liver enzymes, etc.) were compared using two-way ANOVA (with species and diet as main effects) followed by Tukey's honestly significant difference (HSD) and linear regression. We also used *t*-tests to compare the slopes of two regression lines. The data were tested for normality prior to statistical comparisons. To evaluate the dispersion of individuals' values from the mean values within each of the species, we calculated the coefficient of variation (CV=100×s.d./mean; %) of the three *Acomys* species under the two diet treatments. Sample size (*N*=5–6 per species) was kept constant for each of the parameters within each of the diets. For all CV calculations, we used the same sample size; in cases of unequal sample sizes, values from larger groups were removed at random.

In all cases, significance level was set at *P*<0.05. All data were reported as means ± s.e.m. Statistical analysis was conducted using SPSS 19.0 (IBM, Armonk, NY, USA).

RESULTS
Fruit-eating behaviours
Acomys russatus exhibited the 'whole fruit' behaviour (confronter, consumed pulp and seeds mashed together; movie available at: http://y2u.be/RcLDPst87vs) whereas *A. cahirinus* exhibited the 'pulp' behaviour (avoider, consumed the pulp and spat the seeds;

movie available at: http://y2u.be/25XI_mtglPU) (Samuni-Blank et al., 2013). Five individuals of *A. minous* left the fruit untouched, and were not included in the analysis. Interestingly, the most common fruit-eating behaviour of *A. minous* differed from that of the other two *Acomys* species (Table 2). Eight out of 11 individuals employed, at least once, the 'seed' behaviour, by making a hole in the pulp, eating the seeds and leaving the pulp on the cage floor (movie available at: http://y2u.be/yvHL7oA0HbM). The 'seed' behaviour was the dominant behaviour for six of these eight individuals. From these six individuals, four individuals exclusively exhibited the 'seed' (avoider) behaviour and the remaining two individuals alternated between the 'seed' and 'whole fruit' behaviours.

A smaller proportion of *A. minous* (36.6%) preferred the 'whole fruit' (confronter) behaviour (movie available at: http://y2u.be/ghVA7Ibhu8c). Half used the 'seed' strategy exclusively and the others alternated between 'seed' and 'whole fruit' behaviour. Only a single individual used only the 'pulp' (avoider) behaviour (movie available at: http://y2u.be/rb1vC3EbnP8). There was no significant difference between these feeding behaviours (avoider *versus* confronter; *Z*=0.89, *N*=11, n.s.). For all individuals, no intact seeds were found in the feces. Overall, we characterized *A. minous* as avoider because more than 50% of the individuals avoided the consumption of the whole fruit and its activated PSCs.

Physiological effects of fruits
Body mass
Final body mass (% of initial) differed significantly among species ($F_{2,39}$=29.5, *P*<0.001) and between diets ($F_{1,39}$=32.5, *P*<0.001). In addition, there was a marginally significant diet×species interaction ($F_{2,39}$=3.1, *P*=0.05). *Post hoc* Tukey's HSD tests showed that on pulp diet, *A. cahirinus* and *A. russatus* maintained the lowest and highest body mass, respectively (*P*<0.05). Similarly, when fed mash diet, *A. cahirinus* lost significantly more (~20%) of its initial body mass (*P*<0.05), compared with *A. minous* and *A. russatus*, which lost ~15% and ~10%, respectively (Table 3).

Food intake
DM food intake (% body mass day^{-1}) differed significantly among species ($F_{2,39}$=26.4, *P*<0.001) and between diets ($F_{1,39}$=23.9,

Table 3. Summary of the feeding trials: body mass (% of initial), dry matter intake (% body mass day^{-1}) and dry matter digestibility (%) of *A. cahirinus*, *A. minous* and *A. russatus* on day 4 of the trial

Parameter	Diet	*A. cahirinus*	N	*A. minous*	N	*A. russatus*	N
Body mass	Pulp	85.0±1.2[c,d]	8	92.0±2.3[b]	6	99.5±1.3[a]	8
	Mash	81.8±0.7[d]	8	84.8±1.7[c,d]	7	89.2±1.3[b,c]	8
Food intake	Pulp	3.3±0.9[b]	8	5.9±1.0[a,b]	6	8.6±0.5[a]	8
	Mash	0.6±0.2[c]	8	5.3±0.7[b]	7	4.1±0.6[b]	8
Digestibility[n.s.]	Pulp	84.9±2.0	8	82.9±2.1	6	81.3±1.1	8
	Mash	80.3±5.6	6	80.6±2.6	7	81.8±2.0	8

Similar letters adjacent to means indicate no significant difference (*P*>0.05) among the means (two-way ANOVA followed by Tukey's HSD). n.s., not significant. Data are means ± s.e.m.
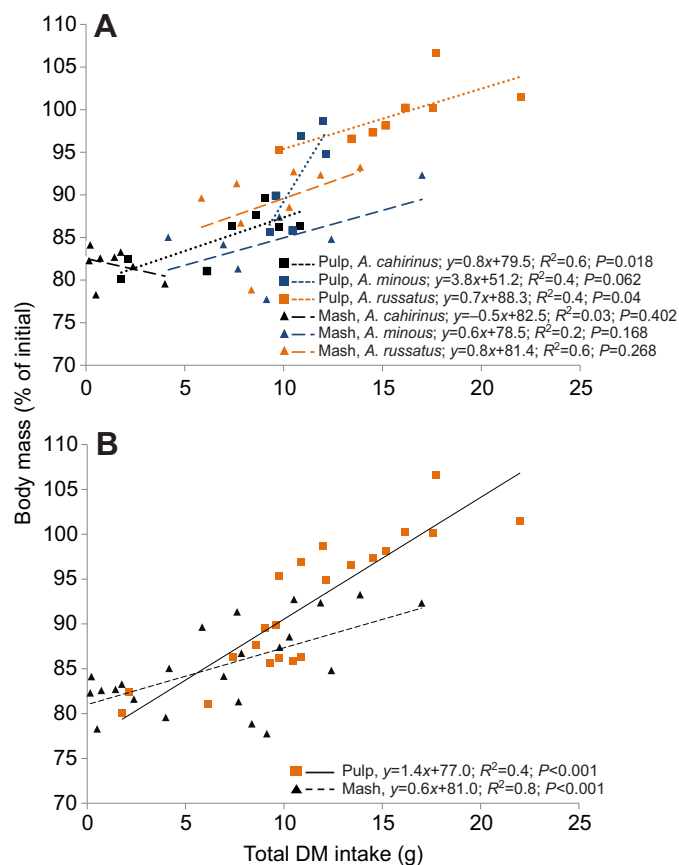
Fig. 1. Change in body mass as a function of total dry matter (DM) intake over all 4 days of the experiment. (A) *Acomys cahirinus* (*N*=16), *A. minous* (*N*=13) and *A. russatus* (*N*=16) feeding on pulp or mash of *Ochradenus baccatus*. (B) Change in body mass as a function of DM intake on the pulp *versus* the mash diet, for all three species combined.

P<0.001). There was a significant interaction between species and diet ($F_{2,39}$=4.1, *P*<0.05). *Post hoc* Tukey's HSD tests showed that *A. minous* maintained DM intake on the mash diet, while the other two species exhibited a significant decrease (*P*<0.05) in DM intake on the mash diet compared with the pulp diet. In addition, *A. cahirinus* DM intake on the mash diet was significantly (*P*<0.05) lower than that of the other two rodents (Table 3).

Digestibility
There were no significant differences in DM digestibility (Table 3) among species ($F_{2,37}$=0.09, n.s.) or between diets ($F_{1,37}$=1.03, n.s.), nor was there a significant diet×species interaction ($F_{2,37}$=0.49, n.s.).

There was a positive correlation between body mass at the end of the trial (% of initial) and total DM food intake for *A. cahirinus* and *A. russatus*, and a marginally positive correlation for *A. minous*, while on the pulp diet (Fig. 1A). There were no significant correlations between intake and final body mass for any species while on the mash diet. The slopes of regression lines differed significantly for pulp and mash diets in all three *Acomys* species combined (t_{43}=3.02, *P*<0.01; Fig. 1B).

Serum biochemical assay
To our knowledge, we were the first to document serum markers of liver damage of any *Acomys* species. Serum ALT activity differed significantly by diet ($F_{1,38}$=7.9, *P*<0.01), but not by species ($F_{2,38}$=0.1, n.s.), and there was a significant diet×species interaction ($F_{2,38}$=3.6, *P*<0.05; Fig. 2A). Inspecting the figure, it was apparent that serum ALT was approximately twice as high in *A. cahirinus* and *A. minous* when eating mash as compared with pulp, whereas *A. russatus* showed no change on the two diets. Despite the significant effects overall in the ANOVA, none of the *post hoc* pair-wise comparisons (Tukey's HSD tests) were significant.

There was a significant difference in serum ALP activity (Fig. 2B) among species ($F_{2,36}$=8.9, *P*=0.001) but not between diets ($F_{1,36}$=1.8, n.s.), nor was there a diet×species interaction ($F_{2,36}$=1.6, n.s.). *Post hoc* Tukey's HSD tests showed that *A. russatus* had significantly lower ALP levels than the other two species (*P*<0.05). All other comparisons were not significant.

For *A. cahirinus* and *A. russatus*, GGT activity was significantly different among species ($F_{1,28}$=11.7, *P*=0.002) but not between diet treatments ($F_{1,28}$=0.8, n.s.), and the diet×species interaction was not significant ($F_{1,28}$=0.4, n.s.). For albumin, total bilirubin and direct bilirubin, there were no significant differences (*P*>0.05) between diet treatments or among species, nor was there a significant diet×species interaction (Table 4).

Coefficient of variation
The CV of all parameters measured for each of the three species and the two diets revealed that for eight out of 10 different CV values, *A. russatus* had the lowest scores (Table 5).

DISCUSSION
The mustard oil bomb products are known to have numerous physiological effects on animals, such as reduction in growth and in food intake, as well as damage to the liver (Duncan and Milne, 1992; Duncan and Milne, 1993; Kim et al., 1997; Sørensen et al., 2001). In the present study, we demonstrated that the activated GLSs of *O. baccatus* differentially affected the performance of seed dispersers and predators.

Two of the three studied species, *A. cahirinus* and *A. russatus*, were previously reported to exhibit very different feeding
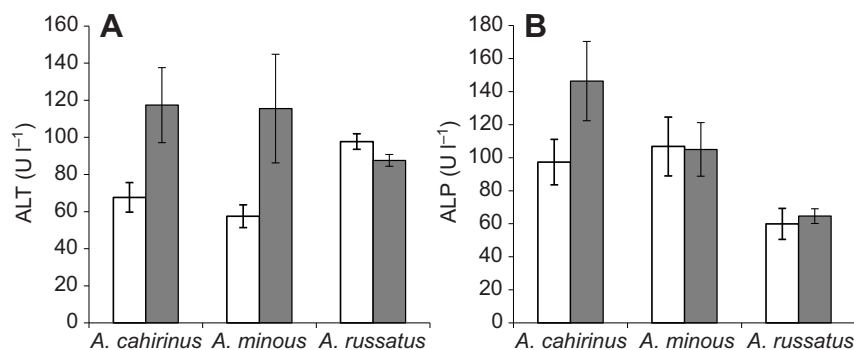

Fig. 2. Serum biochemical assay of *A. cahirinus* (*N*=8 for each diet), *A. minous* (*N*=5–6 for each diet) and *A. russatus* (*N*=8 for each diet) on pulp (white bars) and mash (grey bars) *O. baccatus* fruits for (A) alanine aminotransferase (ALT) and (B) alkaline phosphatase (ALP). Data are presented as means ± s.e.m.

Table 4. Markers of liver damage of *A. cahirinus* (*N*=8 for each diet) *A. russatus* (*N*=8 for each diet) on different diets

Parameter	Diet	*A. cahirinus*	*A. russatus*
GGT (U l^{-1})	Pulp	12.3±1.4	8.9±1.7
	Mash	12.0±0.8	7.0±0.5
Albumin (g dl^{-1})	Pulp	1.1±0.0	1.2±0.1
	Mash	1.1±0.0	1.1±0.0
Total bilirubin (mg dl^{-1})	Pulp	0.1±0.0	0.1±0.0
	Mash	0.1±0.0	<0.05
Direct bilirubin (mg dl^{-1})	Pulp	<0.01	<0.01
	Mash	<0.01	<0.01

GGT, gamma-glutamyl transpeptidase. Data are means ± s.e.m.

Table 5. Coefficient of variation (%) of the three *Acomys* species in the two diet treatments of the different parameters: body mass, dry matter intake, dry matter digestibility, alkaline phosphatase (ALP) and alanine aminotransferase (ALT)

Parameter	Diet	*A. cahirinus*	*A. minous*	*A. russatus*
Body mass	Pulp	3.07	6.15	**1.94**
	Mash	**1.92**	5.92	5.88
Food intake	Pulp	68.93	38.74	**17.41**
	Mash	41.36	**37.18**	45.23
Digestibility	Pulp	7.77	6.29	**2.21**
	Mash	17.04	9.30	**6.38**
ALP	Pulp	29.20	37.38	**11.3**
	Mash	44.82	34.46	**20.57**
ALT	Pulp	40.26	25.95	**13.18**
	Mash	49.50	62.07	**9.87**

The lowest score for each test within each diet is marked in bold.

behaviours: *A. cahirinus* separate the pulp from the seeds to avoid GLS activation while *A. russatus* consume the whole fruit, pulp and seeds. Here, we report that a third species, *A. minous*, evolutionary naïve to *O. baccatus* and closely related to *A. cahirinus*, showed a different dominant behaviour: puncturing a hole in the pulp and consuming only the seeds or consuming the whole fruit. This species tended to avoid the consumption of hydrolyzed GLSs (like *A. cahirinus*), but at the same time acted as a seed predator (like *A. russatus*).

In line with its behaviour, *A. minous* also incurred greater physiological effects than *A. russatus*. The physiological results were consistent with each of the species' fruit-eating strategies: species with a high percentage of PSC avoiders, i.e. individuals spitting seeds or eating only the seeds (Table 2), exhibited a more negative effect of the mash diet on body mass maintenance. After 4 days on the mash diet, *A. russatus* (the confronter) maintained ~90% of its initial body mass, while avoiders *A. minous* and *A. cahirinus* (the seed disperser) maintained only ~85% and ~80% of their initial body mass, respectively. Change in body mass was correlated with food consumption (Fig. 1B) and all species consumed more DM on pulp diet than on mash diet (Table 3).

Although *A. minous* maintained DM intake when feeding on the mash diet, individuals eating that diet lost significantly more body mass than those eating only pulp. This mass loss may stem from the higher energetic costs of detoxifying the hydrolyzed products of the GLSs. Detoxification costs are relatively high, and can be as high as 45% of the basal metabolic costs in specialist herbivores (Sorensen et al., 2005). Accordingly, positive correlations between body mass and food intake for each species were found only on the pulp diet (Fig. 2A). Detoxification costs may also explain the different slopes of the regression lines for the pulp and the mash diets across all *Acomys* species (Fig. 2B).

The very low DM intake of *A. cahirinus*, resulting in significant loss of body mass when feeding on the mash diet, could be explained by the bitter taste of the mustard oil bomb, which serves as a deterrent (Fenwick et al., 1983a; Fenwick et al., 1983b; Garcia-Bailo et al., 2009). Indeed, taste is a valuable tool for estimating food quality, and bitter taste perception prevents animals from ingesting toxic compounds (Chandrashekar et al., 2000; Nelson et al., 2001; Mueller et al., 2005; Chandrashekar et al., 2006). Previous studies have demonstrated that rodents are deterred by bitter taste and also that bitter taste inhibits food intake (Glendinning et al., 1990; Scalera, 1992). However, different mice species may have different taste sensitivities (Glendinning et al., 1990). This could partly explain the higher DM intake values and thus the maintenance of higher body mass of *A. russatus* and *A. minous* when feeding on a mash diet. Also, as individuals of *A. minous* in this study were captured in the wild and thus were exposed to a wider range of diets and

environmental changes, it is reasonable that they will show a greater tolerance compared with captive-bred *A. cahirinus*.

Fleshy ripe pulp is used by plants as a nutritious and easily digestible reward for seed-dispersing animals (Herrera, 1982; Corlett and Lucas, 1990; Jordano, 2000; Izhaki, 2002b). We propose that digestibility did not differ between the pulp and mash diets because of the low percentage of seeds (~8%; wet weight) compared with pulp (~92%) in *O. baccatus* fruits.

As an index of the toxicity of the diets, we tested for serum levels of a set of enzymes and proteins known to increase when hepatobiliary damage occurs [ALT, ALP, GGT, albumin and bilirubin (Ozer et al., 2008)]. Toxins can cause acute liver cell necrosis; upon damage to liver cells, aminotransferases enzymes are released into the bloodstream and their activity is elevated in the serum (Ozer et al., 2008; Hyder et al., 2013). Indeed, for *A. cahirinus* and *A. minous*, ALT levels were almost twofold higher on the mash diet than on the pulp diet. For *A. cahirinus* there was also an increase in ALP levels on the mash diet. However, on mash diet, *A. cahirinus* refused to eat (total consumption over 4 days of the experiment was less than 1.5 g) and lost almost 20% of its initial body mass. Severe weight loss is known to be associated with nonalcoholic fatty liver disease (NAFLD) (Salt, 2004). Mice with NAFLD have been shown to possess significantly higher levels of ALT and ALP, but not albumin (Wang et al., 2011). Thus, the severe weight loss on mash diet can also be the reason for the elevated levels in *A. cahirinus* and *A. minous* (who lost more than 15% of its initial body mass), in which the majority of individuals would typically avoid the consumption of the whole fruit and its activated PSCs.

The seed predator *A. russatus* displayed the least variability (i.e. lowest CVs) out of all the study species while on different diets and within the various parameters. In addition, *A. russatus* appeared more tolerant to *O. baccatus* diets in terms of defending body mass when feeding on the mash diet, which contained the toxic components of the mustard oil bomb. This result is consistent with other studies showing the ability of *A. russatus* to maintain body mass under various conditions (Shkolnik and Borut, 1969; Kam and Degen, 1993; Gutman et al., 2006). Physiologically, the unique adaptive mechanisms of the diurnal *A. russatus* for desert survival (Haim and Borut, 1981; Haim et al., 1994; Haim et al., 2005; Ehrhardt et al., 2005; Levy et al., 2011) may also explain their low variability under the different treatments across various examined parameters.

Despite more than three generations of *O. baccatus* naïvety, the seed predator, *A. russatus*, was the least affected by fruit PSCs,

suggesting its higher physiological tolerance to the activated GLSs, while *A. cahirinus*, the seed disperser, was most negatively affected. The ability of the wild-caught, naïve *A. minous* and the captive-bred *A. cahirinus* to behaviourally circumvent the activation of the GLSs, and the physiological ability of *A. russatus* to tolerate the mustard oil bomb, demonstrate the wide ecological/evolutionary lability of this plant–animal symbiosis to shift from predation to mutualism and *vice versa*.

LIST OF ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
DM	dry matter
GGT	gamma-glutamyl transpeptidase
GLS	glucosinolate
NAFLD	nonalcoholic fatty liver disease
PSC	plant secondary compound

AUTHOR CONTRIBUTIONS

M.S.-B., I.I., M.D.D., W.H.K. and Z.A. conceived the study; M.S.-B., I.I. and M.D.D. designed the study; M.S.-B., M.D.D., K.D.K., W.H.K., P.K. and Z.A. carried out the experiments; M.S.-B., I.I. and W.H.K. analysed the data; M.S.-B., Y.G. and P.L. interpreted the findings. M.S.-B. wrote the paper and all authors contributed substantially to article revision.

COMPETING INTERESTS

No competing interests declared.

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