

1 **Physiological and Behavioural Effects of Fruit Toxins**
2 **on Seed-Predating *versus* Seed-Dispersing Congeneric Rodents**

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18

19 **Keywords:** *Acomys*, Alanine aminotransferase, bitter taste, body mass, fruits,
20 glucosinolates, liver, pulp, secondary compounds, seeds.

21

22 **Summary**

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

43

44 **Introduction**

45 Fleshy, ripe fruit pulp is a nutritious and easily digestible reward that facilitates seed
46 dispersal by animals (Herrera, 1982; Corlett and Lucas 1990; Jordano, 2000; Izhaki,
47 2002a). However, fruits are likely to attract not only seed dispersers but also seed
48 predators since seeds are also a valuable food source (Corlett and Lucas 1990; Kelt et al.
49 2004). Eventually, the nature of the plant-animal interaction is determined by the
50 consumers' fruit eating strategy (Corlett and Lucas 1990; Jordano 2000; Dominy and
51 Duncan 2005).

52 Variability in fruit eating can be a function of multiple parameters, such as age,
53 satiation/hunger, feeding location, seasonality, or availability of other food or water
54 (Levey, 1987; Corlett and Lucas 1990; Gautier-Hion et al. 1993; Kaplin and Moermond
55 1998; Lambert 1999; Samuni-Blank et al. 2012). In addition, fruits may contain plant
56 secondary compounds (PSCs) to deter seed predators or alter the behaviour of the
57 consumer (Cipollini and Levey 1997, Samuni Blank et al. 2012). In folivorous
58 herbivores, evolutionary pressure has resulted in various behavioural and physiological
59 adaptations for coping with PSCs, thus altering plant-herbivore interactions (McArthur et
60 al. 1991; Dearing et al. 2005; Torregrossa et al. 2011). Similar adaptations to fruit PSCs
61 may also influence fruit eating strategies and thus affect plant-animal interactions.

62 Glucosinolates (GLSs) are a common class of fruit PSCs. Intact GLSs are
63 generally nontoxic and have limited effect in defending the plant against herbivores and
64 pathogens. The defensive properties of GLSs are enhanced upon their hydrolysis by the
65 myrosinase enzyme causing the release of a toxic aglycone (Hopkins, 2009). These
66 unstable molecules rearrange into several toxic biological compounds (Kjaer, 1976; Das,
67 2000; Wittstock and Halkier 2002). Generally, GLSs and myrosinases are
68 compartmentalized to prevent toxicity to the plant, and only mix upon damage to tissue
69 (Rask et al. 2000; Hopkins, 2009). The myrosinase-GLS system, also known as the
70 mustard oil bomb, provides plants with an effective defense against generalist herbivores
71 (Wittstock and Halkier 2002).

72 The GLS-myrosinase defense system is employed in the fleshy fruit of
73 *Ochradenus baccatus* (Resedaceae), a common inhabitant of wadis and depressions in
74 Israeli desert regions (Wolf and Shmida 1995, 1997; Wolf and Burns 2001; Bronstein et
75 al. 2007). The fleshy fruits of *O. baccatus* attract a number of consumers because of its
76 high water and sugar content (Bronstein et al. 2007; Spiegel and Nathan 2007; 2011).
77 However, the seeds of *O. baccatus* also represent a valuable nutrient source, as they are
78 rich in protein (~25% of dry mass). To prevent seed predation, the fruits of *O. baccatus*
79 defend themselves with a unique arrangement of the mustard oil bomb, where GLSs are
80 found in the pulp and the myrosinase enzyme is found in the seeds (Samuni-Blank et al.
81 2012). Thus, activation of the mustard oil bomb only occurs if consumers crush both
82 seeds and pulp together.

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

94 The aim of the present study was to test the effects of fruits PSCs (pulp and seeds)
95 on fruit consumers by preventing behavioural circumvention, while testing the
96 physiological impacts of fruit PSCs on seed dispersers versus seed predators. For
97 comparative purposes, we also documented the fruit eating behaviour and physiological
98 responses to fruit PSCs of the Crete spiny mouse (*A. minous*), which is endemic to the
99 Mediterranean island of Crete and evolutionarily naïve to *O. baccatus* fruits. We
100 presented each species with diets containing intact or activated GLSs, and monitored
101 various physiological parameters such as body mass, food intake, dry matter digestibility

102 and serum markers of liver damage. We predicted that the seed predator, *A. russatus*,
103 would be physiologically more adapted to consume activated toxins, as it regularly does
104 so in the wild. We also predicted that consumption of food containing activated GLSs
105 would result in liver damage.

106

107 **Materials and Methods**

108 **Fruit collection**

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

116 **Animals and Maintenance**

117 The experimental protocols were approved by the University of Haifa Committee of
118 Animal Experimentation (Permit number 096/08). *A. cahirinus* (but see Volobouev et al.
119 2007 for a discussion on the *A. cahirinus-dimidiatus* complex) and *A. russatus* were from
120 captive breeding colonies (at least three generations in captivity) originally established
121 from individuals trapped in the vicinity of the Dead Sea (31°28'N, 35°23'E). Animals
122 were maintained at the Department of Biology and Environment at the University of
123 Haifa, Oranim. Adult individuals of *A. minous* were live-trapped in the vicinity of
124 Heraklion, Crete, a few days prior to the experiment using Sherman folding traps placed
125 under rocks. All captured *A. minous* individuals were maintained at the Natural History
126 Museum of Crete (Ministry of Environment, Energy and Climate Change, Permit number
127 117272/586).

128 Prior to experiments, animals were fed rodent chow (Koffolk serial no. 19510) and
129 fresh carrots as a source of free water, *ad libitum*. During the experiments, animals were

130 housed individually in standard mouse cages (21 X 31 X 13 cm) in a temperature-
131 controlled room ($25 \pm 2^{\circ}\text{C}$) under a 12:12 h light-dark cycle. All animals were naïve to *O.*
132 *baccatus* prior to the experiment.

133 **Fruit eating behaviour of *A. minous***

134 To determine the fruit eating behaviour of *A. minous*, we placed each single animal ($n =$
135 16) in a cage with five fruits over-night. The cage floor was examined the next morning
136 for intact fruit parts (pulp or seeds) and the fruit eating behaviour was classified as one of
137 three types: “Whole fruit” - eating pulp and seeds simultaneously and leaving no remains;
138 “Pulp” - eating the pulp and leaving the seeds; “Seed” - eating the seeds and leaving the
139 pulp. Individuals that exhibited more than one fruit eating behaviour were classified
140 according to their dominant strategy. For example, if one individual ate two whole fruits
141 and only the seeds of the remaining three fruits, then the behaviour was classified as
142 “Seed”. We define avoiders as individuals that consume either pulp or seeds, but not
143 both. We define confronters as individuals that consume the whole fruit, pulp and seeds
144 simultaneously. We recorded the number of intact seeds dropped to the cage floor as well
145 as the number intact defecated seeds (in the feces). Similar experiments were previously
146 performed on the two other *Acomys* species (Samuni-Blank et al. 2013).

147 **Physiological Effects of Fruit Toxins**

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

158 Each experiment lasted four days. During the experiment, body mass and food
159 intake were measured daily. Excreta and food leftovers were collected every day from the
160 plastic cage floor, dried (50°C for 24 h), and weighed. Dry matter (DM) digestibility of
161 food consumed by an animal was calculated from its DM food intake and fecal DM
162 output as: $DM \text{ digestibility} = (DM \text{ food intake} - \text{fecal DM output}) / DM \text{ food intake}$.

163 The effect of activated GLSs on the liver was studied through measurements of
164 liver enzymes and liver function test. On the last day of the experiment, animals were
165 euthanized with CO₂ and immediately dissected. Blood samples were collected by heart
166 puncture and centrifuged (15 min at 2500 rpm). Blood serum was collected and stored at
167 -20°C. Samples were analyzed for alkaline phosphatase (ALP) and alanine
168 aminotransferase (ALT). ALT is an intracellular enzyme of hepatocytes and its
169 appearance in blood is indicative of possible liver damage. *Acomys cahirinus* and *A.*
170 *russatus* samples were also analyzed for albumin, gamma-glutamyl transpeptidase
171 (GGT), total bilirubin (Bili. Tot.) and direct bilirubin (Bili. Dir.). Serum levels of all
172 these enzymes and proteins are known to increase when hepatobiliary damage occurs
173 (Ozer et al. 2008). All samples were analyzed at the Laboratory of Clinical Biochemistry
174 in Rambam Medical Center, Haifa.

175 **Statistical analyses**

176 To test for differences between the fruit eating behaviour of *A. minous*, we used a one
177 proportion Z test. Physiological responses on the last day of the diets (body mass, DM
178 digestibility, liver enzymes, etc.) were compared using two-way ANOVA (with species
179 and diet as main effects) followed by Tukey's Honestly-Significant-Difference (HSD) and
180 linear regression. We also used t-test to compare the slopes of two regression lines. The
181 data were tested for normality prior to statistical comparisons. To evaluate the dispersion
182 of individuals' values from the mean values within each of the species, we calculated the
183 coefficient of variation ($CV = 100 * S.D. / \text{mean}, \%$) of the three *Acomys* species under the
184 two diet treatments. Sample size ($n = 5-6$ per species) was kept consistent for each of the
185 parameters within each of the diets. For all CV calculations, we used the same sample

186 size, in cases of unequal sample sizes, values from larger groups were removed at
187 random.

188 In all cases, significance level was set at $P < 0.05$. All data were reported as means
189 \pm standard error (SE). Statistical analysis was conducted using SPSS 19.0 (SPSS, USA).

190

191 **Results**

192 **Fruit eating behaviours**

193 *Acomys russatus* exhibited the "Whole fruit" behaviour (confronter, consumed pulp and
194 seeds mashed together; movie available at: <http://y2u.be/RcLDPst87vs>) whereas *A.*
195 *cahirinus* exhibited the "Pulp" behaviour (avoider, consumed the pulp and spat the seeds;
196 movie available at: http://y2u.be/25XI_mtgIPU) (Samuni-Blank et al. 2013). Five
197 individuals of *A. minous* left the fruit untouched, and were not included in the analysis.
198 Interestingly, the most common fruit eating behaviour of *A. minous* differed from that of
199 the other two *Acomys* species (Table 2). Eight out of eleven individuals employed, at
200 least once, the "Seed" behaviour, by making a hole in the pulp, eating the seeds, and
201 leaving the pulp on the cage floor (movie available at: <http://y2u.be/yvHL7oA0HbM>).
202 The "Seed" behaviour was the dominant behaviour for six of these eight. From these six
203 individuals, four individuals exclusively exhibited the "Seed" (avoider) behaviour and the
204 remaining two individuals alternated between the "Seed" and "Whole fruit" behaviours.

205 A smaller proportion of *A. minous* (36.6%) preferred the "Whole fruit" (confronter)
206 behaviour (movie available at: <http://y2u.be/ghVA7Ibhu8c>). Half used the "Seed"
207 strategy exclusively and the others alternated between "Seed" and "Whole fruit"
208 behaviour. Only a single individual used only the "Pulp" (avoider) behaviour (movie
209 available at: <http://y2u.be/rb1vC3EbnP8>). There was no significant difference between
210 these feeding behaviours (avoider vs. confronter; $Z = 0.89$, $n = 11$, N.S). For all
211 individuals, no intact seeds were found in the feces. Overall, we characterized *A. minous*
212 as avoider since more than 50% of the individuals avoided the consumption of the whole
213 fruit and its activated PSCs.

214 **Physiological Effects of Fruits**

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

222 **Food intake:** DM food intake (% body mass/day) differed significantly among
223 species ($F_{2, 39} = 26.4$, $P < 0.001$) and between diets ($F_{1, 39} = 23.9$, $P < 0.001$). There was a
224 significant interaction between species and diet ($F_{2, 39} = 4.1$, $P < 0.05$). Post-hoc Tukey's
225 HSD tests showed that *A. minous* maintained DM intake on the mash diet, while the other
226 two species exhibited a significant decrease ($P < 0.05$) in DM intake on the mash diet
227 compared to the pulp diet. In addition, *A. cahirinus* DM intake on the mash diet was
228 significantly ($P < 0.05$) lower than that of the other two rodents (Table 3).

229 **Digestibility:** There were no significant differences in DM digestibility (Table 3)
230 among the species ($F_{2, 37} = 0.09$, N.S), between diets ($F_{1, 37} = 1.03$, N.S), and no
231 significant interaction ($F_{2, 37} = 0.49$, N.S.).

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

238 **Serum biochemical assay:** To our knowledge, we were the first to document
239 serum markers of liver damage of any *Acomys* species. Serum alanine aminotransferase
240 (ALT) activity differed significantly by diet ($F_{1, 38} = 7.9$, $P < 0.01$), but not by species ($F_{2,$
241 $38 = 0.1$, N.S), and there was a significant diet*species interaction ($F_{2, 38} = 3.6$, $P < 0.05$)
242 (Fig. 2A). Inspecting the figure, it was apparent that serum ALT was elevated about two

243 times in *A. cahirinus* and *A. minous* when eating mash as compared with pulp, whereas *A.*
244 *russatus* showed no change on the two diets. Despite the significant effects overall in the
245 ANOVA, none of the post-hoc pair-wise comparisons (Tukey's HSD tests) were
246 significant.

247 There was a significant difference in serum alkaline phosphatase (ALP) activity
248 (Fig. 2B) among species ($F_{2, 36} = 8.9$, $P = 0.001$) but not between diets ($F_{1, 36} = 1.8$, N.S)
249 nor was there a diet*species interaction ($F_{2, 36} = 1.6$, N.S). Post-hoc Tukey's HSD tests
250 showed that *A. russatus* had significantly lower ALP levels than the other two species (P
251 < 0.05). All other comparisons were not significant.

252 For *A. cahirinus* and *A. russatus*, gamma-glutamyl transpeptidase (GGT) activity
253 was significantly different among species ($F_{1, 28} = 11.7$, $P = 0.002$) but not between diet
254 treatments ($F_{1, 28} = 0.8$, N.S), and the diet*species interaction was not significant ($F_{1, 28} =$
255 0.4 , N.S). For albumin, total bilirubin (Bili. Tol.) and direct bilirubin (Bili. Dir.), there
256 were no significant differences ($P > 0.05$) between diet treatments, among species or nor
257 was there a diet*species interactions (Table 4).

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

261

262 Discussion

263 The mustard oil bomb products are known to have numerous physiological effects on
264 animals, such as reduction in growth and in food intake, as well as damage to the liver
265 (Duncan and Milne 1992; Duncan and Milne 1993; Kim et al. 1997; Sørensen et al.
266 2001). In the present study, we demonstrated that the activated GLSs of *O. baccatus*
267 differentially affected the performance of seed dispersers and predators.

268 Two of the three studied species, *A. cahirinus* and *A. russatus*, were previously
269 reported to exhibit very different feeding behaviours: *A. cahirinus* separate the pulp from
270 the seeds to avoid the GLSs activation while *A. russatus* consume the whole fruit, pulp

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

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

285 Although *A. minous* maintained DM intake when feeding on the mash diet,
286 individuals eating that diet lost significantly more body mass than those eating only pulp.
287 This mass loss may stem from the higher energetic costs of detoxifying the hydrolyzed
288 products of the GLSs. Detoxification costs are relatively high, and can be as high as 45%
289 of the basal metabolic costs in specialist herbivores (Sorensen et al. 2005). Accordingly,
290 positive correlations between body mass and food intake for each species were found
291 only on the pulp diet (Fig. 2A). Detoxification costs may also explain the different slopes
292 of the regression lines for the pulp and the mash diets across all *Acomys* species (Fig.
293 2B).

294 The very low DM intake of *A. cahirinus*, resulting in significant loss of body mass
295 when feeding on the mash diet, could be explained by the bitter taste of the mustard oil
296 bomb, which serves as a deterrent (Fenwick et al. 1983a,b; Garcia-Bailo et al. 2009).
297 Indeed, taste is a valuable tool to estimate food quality and bitter taste perception
298 prevents animals from ingestion of toxic compounds (Chandrashekar et al. 2000; Nelson
299 et al. 2001; Mueller et al. 2005; Chandrashekar et al. 2006). Previous studies have

300 demonstrated that rodents are deterred by bitter taste and also that bitter taste inhibits
301 food intake (Glendinning et al. 1990; Scalera, 1992). However, different mice species
302 may have different taste sensitivities (Glendinning et al. 1990). This could partly explain
303 the higher DM intake values and thus the maintenance of higher body mass of *A. russatus*
304 and *A. minous* when feeding on a mash diet. Also, since individuals of *A. minous* in this
305 study were captured in the wild and thus were exposed to a wider range of diets and
306 environmental changes, it is reasonable that they will show a greater tolerance compared
307 to captive-bred *A. cahirinus*.

308 Fleshy ripe pulp is used by plants as a nutritious and easily digestible reward for
309 seed dispersing animals (Herrera, 1982; Corlett and Lucas 1990; Jordano, 2000; Izhaki,
310 2002b). We propose that digestibility did not differ between the pulp and mash diets
311 because of the low percentage of seeds (~8%; wet weight) compared to pulp (~92%) in
312 *O. baccatus* fruits.

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

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

339 Despite more than three generations of *O. baccatus* naivety, the seed predator, *A.*
340 *russatus*, was the least affected by fruits PSCs, suggesting its higher physiological
341 tolerance to the activated GLSs, while *A. cahirinus*, the seed disperser, was most
342 negatively affected. The ability of the wild-caught, naïve *A. minous*, and the captive-bred
343 *A. cahirinus* to behaviourally circumvent the activation of the GLSs, and the
344 physiological ability of *A. russatus* to tolerate the mustard oil bomb, demonstrate the
345 wide ecological/evolutionary lability of this plant-animal symbiosis to shift from
346 predation to mutualism and vice versa.

347

348 **List of abbreviations**

349	ALP	Serum alkaline phosphatase
350	ALT	Serum alanine aminotransferase
351	Bil. Dir.	Direct bilirubin
352	Bil. Tot.	Total bilirubin
353	DM	Dry matter
354	GGT	Serum gamma-glutamyl transpeptidase
355	GLSs	Glucosinolates
356	NAFLD	Nonalcoholic fatty liver disease
357	PSCs	Plant secondary compounds

358

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- 523

524 **Legends**

525

526 **Figure 1.** Change in body mass as a function of total dry matter (DM) intake over all
527 four days of the experiment. (A) *A. cahirinus* ($n = 16$), *A. minous* ($n = 13$) and *A.*
528 *russatus* ($n = 16$) feeding on pulp or mash of *O. baccatus*. (B) Change in body mass as a
529 function of dry matter (DM) intake on the pulp vs. the mash diet, combining all three
530 species.

531

532 **Figure 2.** Serum biochemical assay of *A. cahirinus* ($n = 8$ for each diet), *A. minous* ($n =$
533 $5-6$ for each diet) and *A. russatus* ($n = 8$ for each diet) on pulp (white bars) and mash
534 (gray bars) *O. baccatus* fruits for (A) Alanine aminotransferase (ALT) and (B) Alkaline
535 phosphatase (ALP). Data are presented as means \pm SE.

536

537 **Table 1.** Diet components (%).

538

539 **Table 2.** Summary of the fruit-eating behaviour of the three congeneric species of
540 *Acomys*. *5 other individuals did not consume any part of the fruit and were excluded.

541

542 **Table 3.** Summary of the feeding trials. Body mass (% of initial), dry matter intake (%
543 body mass/day) and dry matter digestibility (%) of *A. cahirinus*, *A. minous*, and *A.*
544 *russatus* on day 4 of the trial. Similar letters adjacent to means indicate no significant
545 difference ($P > 0.05$) among the means (two-way ANOVA followed by Tukey's HSD).
546 Data are means \pm SE.

547

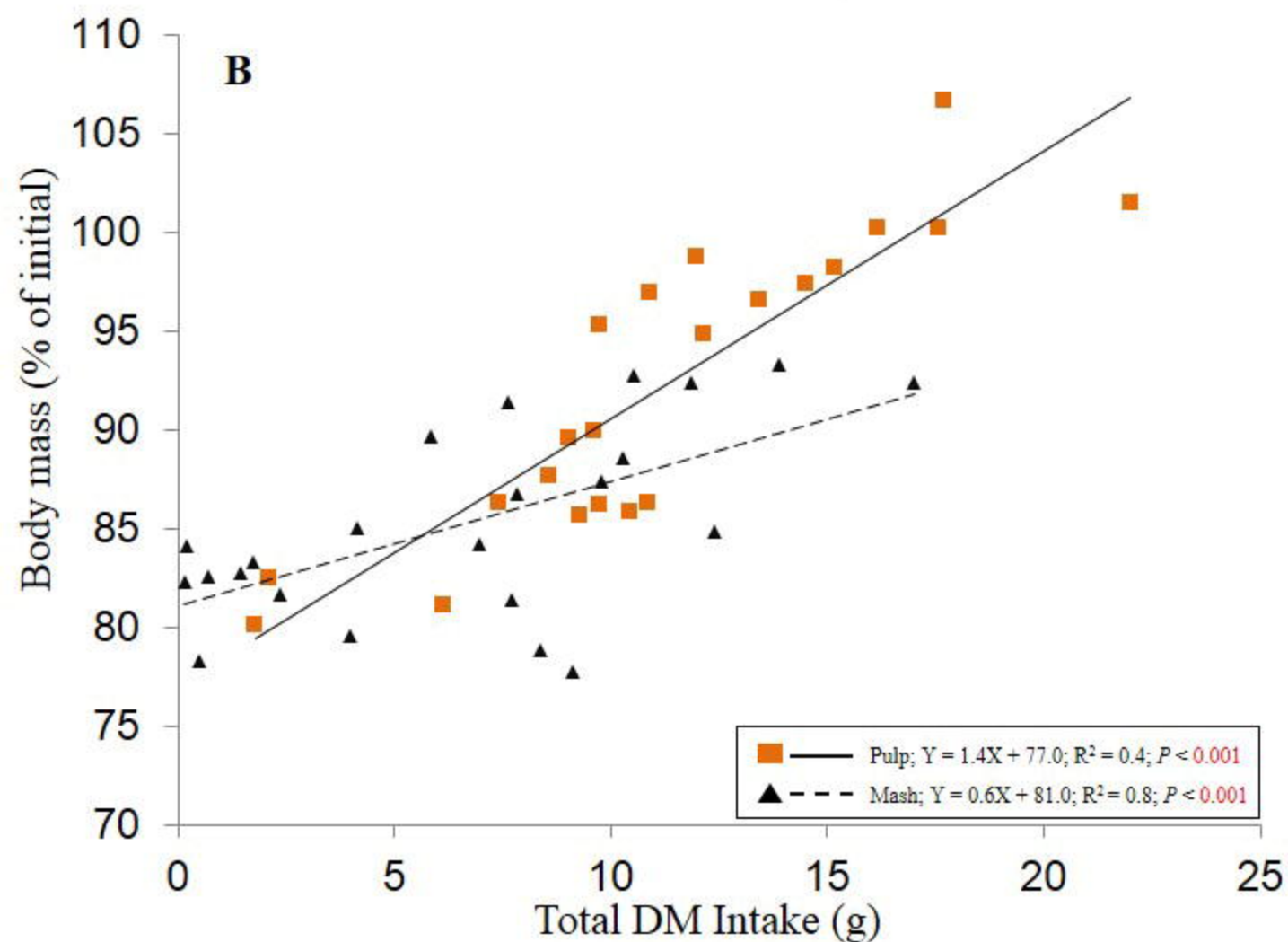
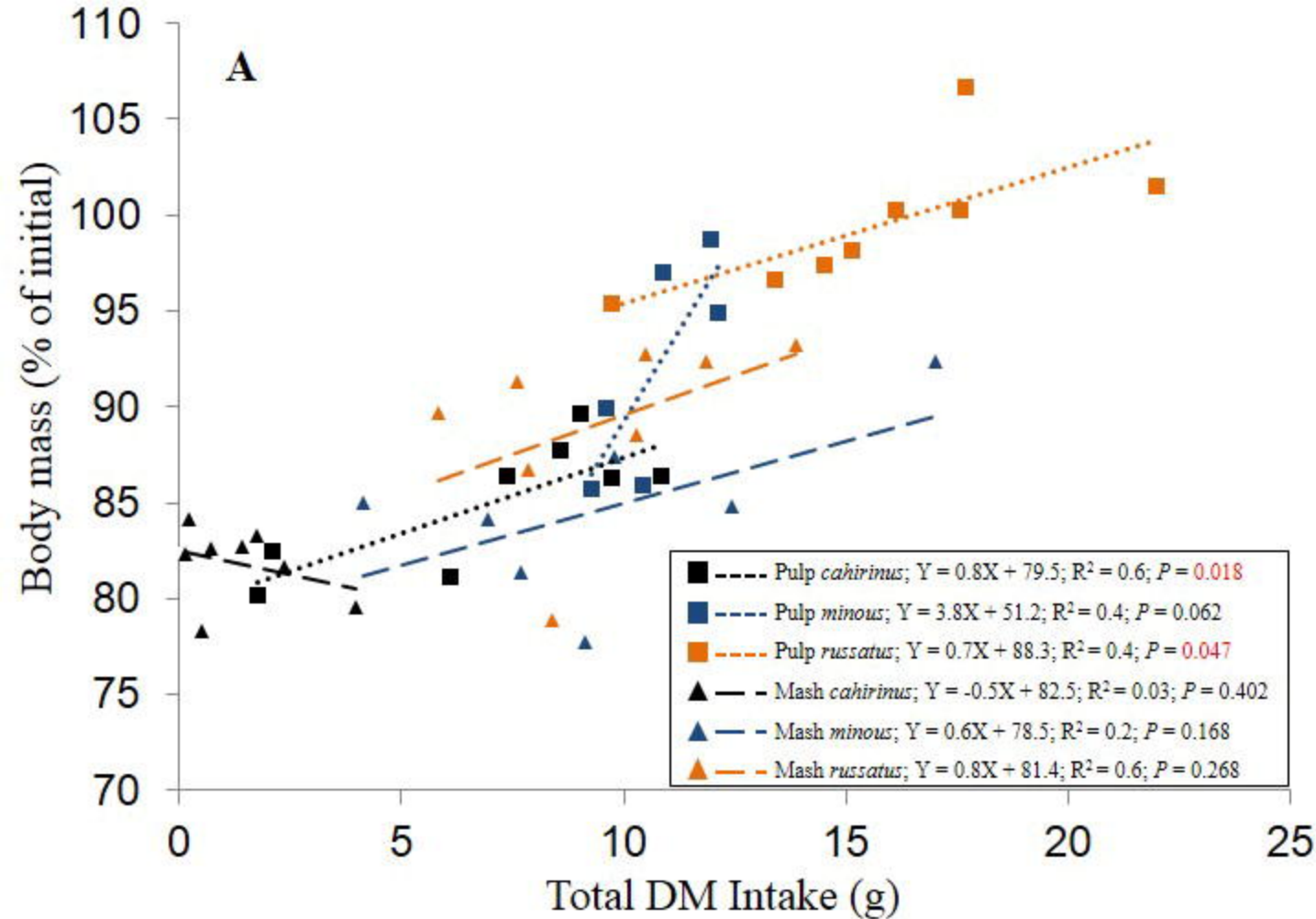
548 **Table 4.** Markers of liver damage of *A. cahirinus* ($n = 8$ for each diet) *A. russatus* ($n = 8$
549 for each diet) on different diets. The parameters measured are gamma-glutamyl

550 transpeptidase (GGT), albumin, total bilirubin (Bili. Tol.) and direct bilirubin (Bili. Dir.)

551 Data are means \pm SE.

552

553 **Table 5.** Coefficient of variation (%) of the three *Acomys* species in the two diet
554 treatments of the different parameters: body mass, dry matter intake, dry matter
555 digestibility, alkaline phosphatase (ALP) and alanine aminotransferase (ALT). The
556 lowest score for each test within each diet is marked in bold.



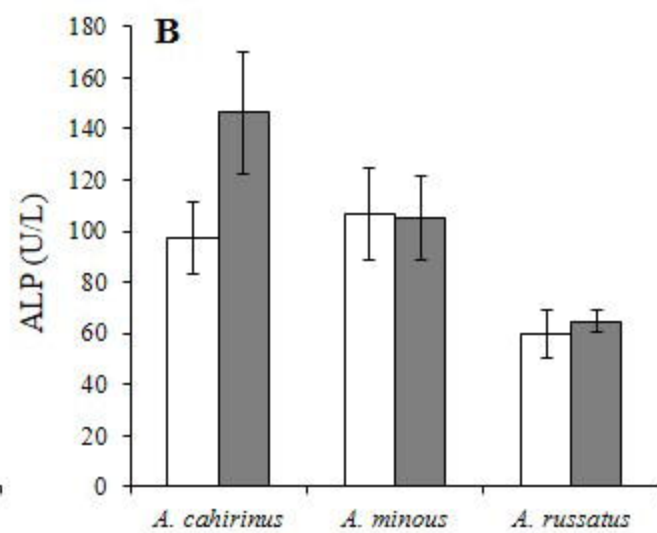
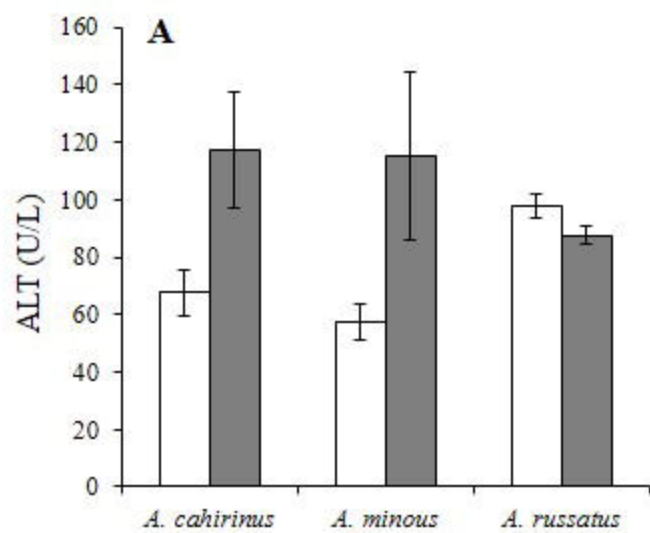


Table 1.

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

Table 2.

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

Table 3.

Parameters	Diet	<i>A. cahirinus</i>	N	<i>A. minous</i>	N	<i>A. russatus</i>	N
Body Mass	Pulp	85.0 ± 1.2 ^{CD}	8	92.0 ± 2.3 ^B	6	99.5 ± 1.3 ^A	8
	Mash	81.8 ± 0.7 ^D	8	84.8 ± 1.7 ^{CD}	7	89.2 ± 1.3 ^{BC}	8
Food intake	Pulp	3.3 ± 0.9 ^B	8	5.9 ± 1.0 ^{AB}	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

Table 4.

Parameters	Diet	<i>A. cahirinus</i>	<i>A. russatus</i>
GGT (U/L)	Pulp	12.3 ± 1.4	8.9 ± 1.7
	Mash	12.0 ± 0.8	7.0 ± 0.5
Albumin (g/dL)	Pulp	1.1 ± 0.0	1.2 ± 0.1
	Mash	1.1 ± 0.0	1.1 ± 0.0
Bili. Tot. (mg/dL)	Pulp	0.1 ± 0.0	0.1 ± 0.0
	Mash	0.1 ± 0.0	< 0.05
Bili. Dir. (mg/dL)	Pulp	< 0.01	< 0.01
	Mash	< 0.01	< 0.01

Table 5.

Parameters	Diet	Coefficient of variation (%)		
		<i>A. cahirinus</i>	<i>A. minous</i>	<i>A. russatus</i>
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