

RESEARCH ARTICLE

Cheating on the mutualistic contract: nutritional gain through seed predation in the frugivorous bat *Chiroderma villosum* (Phyllostomidae)

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ABSTRACT

Most frugivorous bats are efficient seed dispersers, as they typically do not damage seeds and transport them over long distances. In contrast, bats of the phyllostomid genus *Chiroderma* cheat fig trees by acting more as seed predators than as seed dispersers. The bats initially separate seeds from fruit pulp in the mouth. After extracting the juice from the fruit pulp, they thoroughly chew the seeds and spit out small seed fragments in a pellet. Consequently, the faeces contain almost no viable seeds. We compared the nutrient content of intact fig seeds with ejecta and faecal samples from both *Chiroderma villosum* and the ‘conventional’ frugivorous bat *Artibeus watsoni*. We show that *C. villosum* can extract nutrients from the seeds, especially protein and fat. The processing time of figs showed no significant difference between the two bat species. Food-choice experiments showed that *C. villosum* preferred fig species with more seeds over those with fewer seeds. This preference, in combination with the specialized seed-chewing behaviour, leads to an increased nutrient intake per fig. This unique strategy enables *C. villosum* to satisfy its nutritional requirements with a lower number of figs than other species, which decreases the amount of energy necessary for foraging flights as well as the predation risk during foraging.

KEY WORDS: Feeding behaviour, Nutrient, Parasite, Mutualism, Fig-eating bat

INTRODUCTION

Around 550 plant species in the Neotropics have so far been found to depend on bats as seed dispersers (Lobova et al., 2009). In this mutualistic relationship, bats obtain nutrients from fruits and the plants profit through the seed dispersal services of the frugivores (Ridley, 1930; Medellín and Gaona, 2006; Henry and Jouard, 2007). Most fig-eating bats are very effective seed dispersers. They select ripe fruits, transport them from the mother tree to a feeding roost where they process them safe from predators (Morrison, 1978), and finally egest up to 80% of viable, undamaged seeds (Morrison, 1980). Frugivorous bats typically take a bite of a fruit and press the tissue with their strong tongue against the ridged palate (Shanahan et al., 2001), squeezing out and swallowing the fruit juice, usually together with a considerable number of seeds. The remains of the fruit pulp, mostly consisting of non-digestible fibrous material, are then spat out.

Bats of the genus *Chiroderma* demonstrate a very different behaviour. They first separate fruit pulp and seeds in their mouth. After extracting the fruit juice from the pulp material, they spit out an almost dry fruit fibre pellet. Then they begin to chew the accumulated seeds and spit out a second pellet, consisting mostly of crushed seed remains. Consequently, their faeces contain almost no viable seeds (Nogueira and Peracchi, 2003). Nogueira and Peracchi (2003) proposed that *C. doria* and probably also *C. villosum* Thomas 1891 act more as seed predators than as dispersers; however, they had only a very small sample size from *C. villosum*. To verify whether seed predation is a regular foraging behaviour of this species, and to better understand the ecological and evolutionary relevance of different forms of fruit consumption, we observed *C. villosum* eating figs and predicted that, because of the similar cranial morphology (Nogueira et al., 2005) and dentition, this species should also act as a seed predator. We also predicted that as a result of the additional seed-chewing behaviour, *C. villosum* should spend more time processing a fig than conventional fig-eating bats that swallow and distribute the seeds, such as *Artibeus watsoni* Thomas 1901.

This unique seed-chewing behaviour has been suspected to contribute to an enhanced acquisition of nutrients (Nogueira and Peracchi, 2003) but the actual effect had not been quantified. Figs are often classified as ‘nutrient-poor’ fruits because of their low protein and lipid content (Snow, 1971), though this may not be a universal feature (Milton, 2008). There has been some debate about whether fig-eating bats can obtain enough protein by just consuming figs or whether they have to supplement their diet with insects, pollen or leaves to keep a positive protein balance (Fleming et al., 1972; Gardner, 1977; Thomas, 1984; Herbst, 1986; Kunz and Diaz, 1995; Herrera et al., 2001; Albrecht, 2012). As fig seeds contain high concentrations of lipids and proteins (Morrison, 1980; Wendeln et al., 2000), we hypothesized that chewing of seeds may enhance the acquisition of nutrients, especially of proteins and lipids. We predicted that *C. villosum* should gain more nutrients from a fruit than would more conventionally behaving fig-eating bats such as *Artibeus watsoni*.

RESULTS

Processing time and chewing movements

The small fig-eating bat *A. watsoni* (12 g) invested on average 1 h to eat a fig (7–9 g) (mean±s.d., 58.94±7.79 min, $N=5$). The larger *C. villosum* (22 g) completed the same task in about the same time (52.78±10.72 min, $N=7$). After standardizing processing time for body and fig mass, complete processing of a fig was longer for *C. villosum* (146.14±63.42 min, $N=7$) than for *A. watsoni* (96.76±16.97 min, $N=5$); however, this difference was not significant (independent samples t -test: $N_{CV}=7$, $N_{AW}=5$, $t_{10}=1.68$, $P=0.12$). After processing the fruit pulp, all *C. villosum* showed their unique seed-chewing behaviour; this was never observed in *A. watsoni*.

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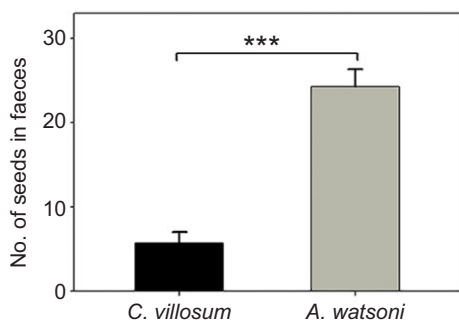


Fig. 1. Mean number of seeds in single faeces from *Chiroderma villosum* and *Artibeus watsoni*. Values are means \pm 1 s.e.m., *** P \leq 0.001.

Mastication movements of *C. villosum* were much faster when chewing seeds (229.42 ± 14.70 movements min^{-1} , $N=7$) than when chewing pulp (147.30 ± 21.15 movements min^{-1} , $N=7$) (paired samples t -test: $N_{\text{pulp}}=7$, $N_{\text{seed}}=7$, $t_6=-7.35$, $P<0.001$). Seed mastication movements by *C. villosum* were also faster than pulp mastication movements by *A. watsoni* (189.30 ± 32.07 movements min^{-1} , $N=5$); however, *C. villosum* chewed pulp slower than *A. watsoni* (independent samples t -test: $N_{\text{Aw}}=5$, $N_{\text{Cv}}=7$, $t_{10}=13.64$, $P=0.015$). Seed chewing was always accompanied by clearly audible cracking sounds (supplementary material Movie 1).

When measuring mastication duration, we found that *C. villosum* chewed three times longer on the seeds (105.19 ± 19.14 s, $N=7$) than on pulp (32.41 ± 8.49 s, $N=7$) (paired samples t -test: $N_{\text{pulp}}=7$, $N_{\text{seed}}=7$, $t_6=12.19$, $P<0.001$). Seed chewing by *C. villosum* also lasted longer than pulp chewing by *A. watsoni* (14.13 ± 3.19 s, $N=5$) (independent samples t -test: $N_{\text{seed,Cv}}=7$, $N_{\text{pulp,Aw}}=5$, $t_{10}=10.36$, $P<0.001$). *Chiroderma villosum* also chewed each pulp pellet longer than *A. watsoni* (independent samples t -test: $N_{\text{pulp,Aw}}=5$, $N_{\text{pulp,Cv}}=7$, $t_{10}=4.56$, $P=0.001$).

Seed predation

The pulp ejected by *C. villosum* after initial chewing contained significantly fewer seeds per unit dry mass (1.52 ± 0.46 seeds 0.5 g^{-1} pulp, $N=9$) than that from *A. watsoni* (28.51 ± 6.07 seeds 0.5 g^{-1} pulp, $N=5$) (independent samples t -test: $N_{\text{Cv}}=9$, $N_{\text{Aw}}=5$, $t_{12}=6.00$, $P<0.001$).

Faecal material of *C. villosum* contained significantly fewer intact seeds (5.76 ± 4.30 seeds, $N=12$) than that of *A. watsoni* (24.30 ± 4.65 seeds, $N=5$) (independent samples t -test: $t_{15}=7.90$, $P<0.001$) (Fig. 1).

Chemical composition

Seeds of *Ficus obtusifolia* contained a high amount of nutrients, including a significantly higher concentration of lipids than the fruit pulp (see Table 1). Similar differences were found for *Ficus nymphiifolia* but the low sample size prohibited statistical analyses

for this species. Therefore, we restricted further analyses to *F. obtusifolia*. Nutrient analysis of *F. obtusifolia* fruits showed that the seeds contained $70.23 \pm 6.81\%$ of the lipids ($N=10$), $31.28 \pm 6.20\%$ of the soluble protein ($N=10$) and $40.58 \pm 7.76\%$ of the nitrogen ($N=10$) of a fig. Sugars are mainly contained in the pulp (90.06%), with only a small amount in the seeds ($9.94 \pm 1.88\%$, $N=10$) (Fig. 2).

Nutrient gain

By comparing the nutrient content of chewed seed remains with that from intact seeds, we found that *C. villosum* extracted a major proportion of the nutrients available in seeds. On average, they extracted $89.06 \pm 6.24\%$ of the lipids ($N=8$), $91.34 \pm 3.05\%$ of the soluble protein ($N=8$), $84.66 \pm 5.89\%$ of the sugar ($N=8$) and $57.82 \pm 7.89\%$ ($N=8$) of the nitrogen present in the seeds (Fig. 3).

Paired t -tests showed that the nutrient gain from pulp was significantly lower than that from seeds for most nutrients, except for sugar (lipids: $t_7=13.25$, $P<0.001$; nitrogen: $t_7=8.13$, $P<0.001$; soluble protein: $t_7=24.28$; $P<0.001$; sugar: $t_7=2.27$; $P=0.058$). Bats extracted $29.02 \pm 12.77\%$ of the lipids in the pulp ($N=8$), $33.37 \pm 7.41\%$ of the soluble protein ($N=8$) and $21.80 \pm 9.64\%$ of the nitrogen ($N=8$). They extracted similar proportions of sugar from pulp and seeds ($89.26 \pm 3.86\%$ and $84.66 \pm 5.89\%$, $N=8$). Extraction of lipids, nitrogen and soluble protein was significantly lower from pulp than from seeds (paired t -test, all $P<0.001$; Table 2).

Per fig, bats obtained on average 0.009 g lipids from pulp and 0.06 g from seeds. By additionally chewing seeds, *C. villosum* extracted seven times more lipids per fig (paired t -test: ± 0.02 s.d.; $N_{\text{pulp}}=8$, $N_{\text{seeds}}=8$, $t_7=-8.06$, $P<0.001$) than the non-seed-chewing *A. watsoni*. Through seed chewing, *C. villosum* was able to double the amount of soluble protein and nitrogen extracted per fruit. As the bats were eating a mean of seven figs per night, they obtained an extra $\sim 0.4 \text{ g}$ lipids per night through seed chewing. Sugar, in contrast, was obtained in significantly higher amounts from pulp than from seeds (Fig. 3, Table 2; paired t -test: ± 0.16 s.d.; $N_{\text{pulp}}=8$, $N_{\text{seeds}}=8$, $t_7=7.00$, $P<0.001$).

Because of problems in obtaining a sufficient number of ripe fruits for comparison of nutrient assimilation, we obtained data for only two individuals of *A. watsoni*, while we could perform the experiments with eight *C. villosum*. Efficiency of nutrient extraction was similar in the two species (Table 2).

Chiroderma villosum metabolized the swallowed nutrients very efficiently and lost only very small quantities through the faeces: less than 2% of each nutrient were lost through faecal material (lipids: $1.28 \pm 2.14\%$, $N=8$; protein: $1.97 \pm 1.07\%$, $N=8$; sugar: $1.87 \pm 0.91\%$, $N=8$), except for nitrogen ($28.70 \pm 5.78\%$, $N=8$).

Seed content and fruit-choice experiment

We caught all bats for our experiments near *F. obtusifolia* and *F. nymphiifolia* trees, and figs from several of these trees (*F. obtusifolia* $N=10$; *F. nymphiifolia* $N=4$) were accepted by all *C. villosum* ($N=17$). Figs from *Ficus popenoi* ($N=2$) and *Ficus citrifolia* ($N=1$) trees were

Table 1. Macronutrient concentrations in seeds and pulp of *Ficus obtusifolia* ($N=10$) and *Ficus nymphiifolia* ($N=2$) fruits

Species	Fig part	Lipids	Soluble protein	Nitrogen	Soluble sugar
<i>F. obtusifolia</i>	Pulp	0.0285 ± 0.0023	0.0866 ± 0.0032	0.0097 ± 0.0007	0.5310 ± 0.0548
	Seeds	0.0687 ± 0.0053	0.0417 ± 0.0035	0.0065 ± 0.0004	0.0566 ± 0.0055
t -value		7.72	9.25	3.35	9.32
P -value		<0.001	<0.001	0.009	<0.001
<i>F. nymphiifolia</i>	Pulp	0.0252 ± 0.0060	0.0879 ± 0.0033	0.0056 ± 0.0001	0.3159 ± 0.0059
	Seeds	0.0554 ± 0.0124	0.0277 ± 0.0081	0.0046 ± 0.0011	0.0383 ± 0.0105

Values (means \pm 1 s.e.m.) are in g per dry mass of fruit. t - and P -values indicate differences between pulp and seeds according to paired t -tests; statistical analyses were not possible for *F. nymphiifolia* because of the small sample size.

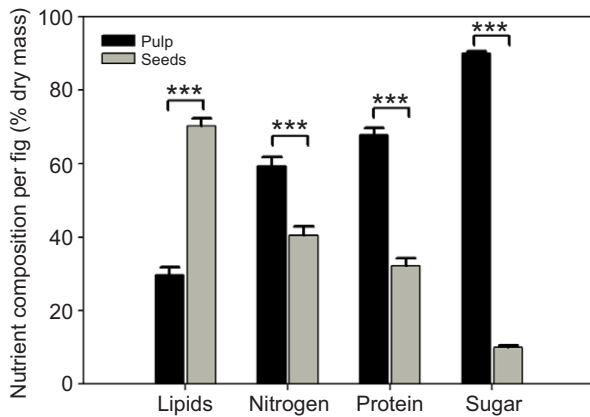


Fig. 2. Nutrient distribution between pulp and seeds in *Ficus obtusifolia* fruits. Values are means+1 s.e.m., *** $P \leq 0.001$. 'Protein' corresponds to soluble protein.

accepted by all three individuals to which they were presented. The tested bats each ate at least five fruits of these species, even when they were also offered fruits from *F. obtusifolia*. In the case of *F. citrifolia*, *C. villosum* was able to perform the seed-chewing behaviour in spite of the extremely small fruits (1 g) and seeds. Wendeln et al. (2000) also captured 7% of *C. villosum* ($N=23$) at *F. popenoi* and found seeds of *F. citrifolia* in one faecal sample. Because of this we defined *F. obtusifolia*, *F. nymphiifolia*, *F. popenoi* and *F. citrifolia* as 'accepted'.

During fruit-choice experiments, we presented ripe figs from *Ficus insipida* ($N=3$) trees together with *F. obtusifolia* fruits to three *C. villosum*. During three nights, none of the *F. insipida* figs were accepted (paired t -test: $t_2=5.50 \pm 1.16$ s.d.; $N_{insipida}=3$, $N_{obtusifolia}=3$, $P=0.032$). We observed the same situation when offering figs from *Ficus maxima* ($N=1$). We therefore categorized fruits from *F. insipida* and *F. maxima* as 'rejected'.

'Accepted' figs contained significantly more seeds than the 'rejected' ones (independent samples t -test: $t_{34}=3.63$; $P < 0.001$) (Fig. 4). In 'accepted' figs, seeds accounted for $27.79 \pm 8.82\%$ of the dry mass of the fruit ($N=26$); in 'rejected' figs, seeds were only $16.68 \pm 6.31\%$ dry mass ($N=10$).

However, 'accepted' fruits did not have significantly heavier seeds than 'rejected' fruits (independent samples t -test: $t_{34}=1.922$, $P < 0.063$). The mass of 10 seeds from 'accepted' figs was 0.018 ± 0.008 g ($N=25$); from 'rejected' fruits it was 0.013 ± 0.002 g ($N=11$).

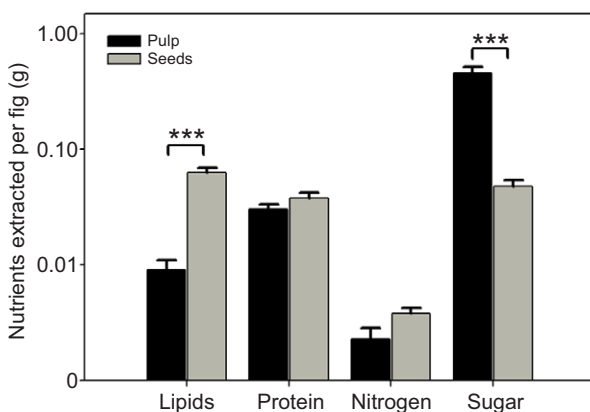


Fig. 3. Nutrients ingested by *C. villosum* from pulp and seeds per fig. Values are means+1 s.e.m. of log-transformed data. *** $P \leq 0.001$.

DISCUSSION

Our experiments clearly confirm that *C. villosum* are more seed predators than seed dispersers, as demonstrated by the low number of intact seeds in faeces and ejected pulp pellets. *Chiroderma villosum* dispersed only 15% of the seeds scattered by *Artibeus watsoni*. We could not show that the seed-predator *C. villosum* invested more time in processing a fig than a standard frugivorous bat, as was first proposed by Nogueira and Peracchi (2003). Nevertheless, our results matched the value reported for *C. villosum* of an average of 45 min to process a 7 g fig (Nogueira and Peracchi, 2003). After applying our standardization for body and fig mass, this corresponds to 141.3 min, which is very similar to our value of 146.1 min. A larger sample size and the inclusion of more species are probably needed to definitively answer the question of whether seed-predating bats have a longer fig-processing duration than other fig-eating bats.

Irrespective of processing time, *C. villosum* received distinct nutritional benefits through processing and consumption of seeds in addition to fig fruit pulp. Our results confirm the suggestions of Nogueira and Peracchi (2003) that through the chewing of seeds, *C. villosum* obtained more nutrients from a fig than obtained by more conventionally behaving frugivorous bats. *Chiroderma villosum* gain seven times more lipids per fig and can double the amount of extracted protein and nitrogen. While 70% of the total lipids of a fig and 30–40% of protein and nitrogen are stored within seeds, most other frugivorous bats do not utilize these resources. The longstanding question (Fleming et al., 1972; Gardner, 1977; Thomas, 1984; Herbst, 1986; Kunz and Diaz, 1995; Herrera et al., 2001; Albrecht, 2012) of whether frugivorous bats are able to gain enough proteins by just feeding on figs seems to be answered at least for *C. villosum*.

Chiroderma villosum extracted nearly 90% of all nutrients from the seeds, with the exception of sugar. This high efficiency seems to be mediated through the very intense seed chewing. These bats chew twice as long on a portion of seeds than on pulp, while using nearly twice the number of chewing movements per minute compared with fruit pulp mastication. That *C. villosum* were slower to chew a piece of pulp than the smaller *A. watsoni* is probably explained by the fact that *C. villosum* is simultaneously separating seeds and pulp in the mouth.

This extraordinary behavioural difference is also reflected in specific food preferences. All fruits eaten by *C. villosum* in this study (*F. nymphiifolia*, *F. obtusifolia*, *F. popenoi*, *F. citrifolia*) and also in the study by Nogueira and Peracchi (2003) (*Ficus tomentella*, *Ficus cyclophylla*) were strangler figs (*Ficus*, subgenus *Urostigma*) that use other trees as structural support. This observation is confirmed by Wendeln et al. (2000), who captured 37% of their *C. villosum* bats at fruiting *F. nymphiifolia* and 28% at fruiting *F. obtusifolia* ($N=328$ bats captured in total). In contrast, the 'rejected' fig species (*F. insipida* and *F. maxima*) are free-standing figs (subgenus *Pharmacosycea*). The seeds of the subgenera *Urostigma* and *Pharmacosycea* differ distinctly in morphology. Seeds from the strangler figs are surrounded by a gelatinous coating that swells up when in contact with water, thus making them 'slippery' (Heer et al., 2010; I.W., unpublished observations), while seeds from free-standing figs lack such a coating. Strangler fig trees develop initially as epiphytes, and this gelatinous coating probably functions to attach the seeds to a tree trunk during dispersal. As *C. villosum* rejects figs with non-slippery seeds, we propose that the gelatinous seed coating of strangler figs is important in the separation of pulp and seeds by *C. villosum*. This separation works very efficiently, as demonstrated by the near-absence of seeds in pulp

Table 2. Percentage of nutrients extracted and amount of nutrients consumed per fig from pulp by *Artibeus watsoni* (N=2) and from pulp and seeds by *Chiroderma villosum* (N=8)

Nutrients	<i>A. watsoni</i>		<i>C. villosum</i>			
	Fruit pulp		Fruit pulp		Seeds	
	% Extracted from pulp	Mass extracted from pulp (g)	% Extracted from pulp	Mass extracted from pulp (g)	% Extracted from seeds	Mass extracted from seeds (g)
Lipids	27.65±6.63	0.0137±0.0063	29.02±12.77	0.0090±0.005	89.06±6.24	0.0628±0.020
Nitrogen	15.03±0.36	0.0013±0.0000	21.18±9.64	0.0023±0.002	57.82±7.89	0.0038±0.001
Protein	21.33±2.31	0.0165±0.0023	33.37±7.41	0.0303±0.009	91.34±3.05	0.0380±0.012
Sugar	76.06±0.10	0.4497±0.0004	89.26±3.86	0.4595±0.173	84.66±5.89	0.0712±0.026

pellets of *C. villosum*. Similarly, Uzzurum and Heideman (1991) observed that fig-eating pteropodid bats swallowed mainly viable seeds with the slippery coating while seeds damaged by developing fig wasps had no such coating and were spat out together with the fruit pulp ejecta.

This mechanical facilitation might explain why *C. villosum* generally prefers fruits from strangler fig species over those from free-standing figs, even if the latter contain seeds of similar mass. The separation of seeds aided by the slippery coating is probably more efficient than consuming figs with larger seeds from free-standing *Pharmacosycea* species that lack this coating. This assumption is supported by the observation that *C. villosum* do not feed on free-standing *F. insipida* and *F. maxima*, although they are very common fig species on Barro Colorado Island (Handley et al., 1991; Albrecht, 2012) and therefore easier to find than the stranglers *F. nymphiifolia* or *F. obtusifolia*.

Within the strangler figs, we observed a preference of *C. villosum* for species with more seeds such as *F. nymphiifolia* and *F. obtusifolia*. In combination with their specialized feeding behaviour, this preference leads to an increased nutrient intake per fig. This enables *C. villosum* to satisfy its daily nutritional requirements with a lower number of figs (Nogueira and Peracchi, 2003) and reduces the number of energetically expensive foraging flights that involve carrying fruits that may weigh up to 36% of their body mass. Additionally, this decreases the exposure to predation during foraging flights (Kalko et al., 1996b).

Typically, a mutualistic system remains in equilibrium if the density of the cheaters and overall cost caused to their respective partners is rather low (Bronstein, 2001a; Ferriere et al., 2002; Bronstein et al., 2003; Aizen et al., 2014). Evolutionary pressure on fig trees to protect their seeds against the seed-predating *C. villosum* seems to be comparatively low, at least at our study site. Barro Colorado Island harbours 20 species of frugivorous bats, of which 10 are considered to be fig specialists (Kalko et al., 1996a; Giannini

and Kalko, 2004; Kalka et al., 2008). Among these, *C. villosum* is the only seed-predating species and is rather rare compared with regular frugivorous bats, such as *Artibeus jamaicensis* (Handley et al., 1991; Kalko et al., 1996a). A long-term study on Barro Colorado Island showed a ratio of only one captured individual of *C. villosum* per 35 of the larger *A. jamaicensis* (Handley et al., 1991). Additionally, bat–fruit networks seem to be highly cohesive and robust mutualistic systems. Simulated cumulative removal of bat or plant species does not lead easily to the breakdown of the system, as most fig species are dispersed by several bat species and so there is enough functional redundancy to keep the system functional, even when losing a major dispersal species (Mello et al., 2011). This indicates that a single cheating, seed-predating bat species has only a very low destabilizing impact on the system. The actual evolutionary pressure on fig trees to escape seed predation by bats, e.g. through protecting their seeds by a thicker seed coat or through toxins, should therefore be rather low. Indeed, in the highly specialized fig pollination system, defensive secondary plant compounds in ovaries and seeds might also affect the important aganoid wasps that, while pollinating, also deposit their eggs in and feed on certain ovaries (Janzen, 1979). As seed dispersal is generally a more diffuse interaction than pollination, it is more difficult for plants to develop mechanisms that restrict access to fruits (Mello et al., 2011).

The results of this study on the feeding behaviour of *C. villosum* emphasize that the degree of mutual benefits may vary widely among different bat–plant interactions. It is probable that a continuum exists in these ‘uneasy partnerships’ (Howe and Westley, 1988; Bronstein, 2001b), ranging from predation to mutualism, and a fruiting tree can tolerate ‘cheating’ agents as long as these are not too common in relation to conventional mutualists.

MATERIALS AND METHODS

Our study was conducted on Barro Colorado Island, a field station of the Smithsonian Tropical Research Institute in Panama (9°15′–9°20′N, 79°54′–79°55′W) from March 2008 to June 2010. The study was conducted in accordance with all national laws of Panama and covered by a research permit of Autoridad Nacional del Ambiente (ANAM) and by the Animal Care Committee (IACUC) of the Smithsonian Tropical Research Institute. Bats were captured under fruiting trees of *F. obtusifolia* and *F. nymphiifolia*. Figs from both tree species are commonly eaten by both *C. villosum* and *A. watsoni* (Wendeln et al., 2000). Bat species were identified using an unpublished key to the bats of the lowlands of Panamá by C. O. Handley, Jr and E.K.V.K.

We conducted our experiments with adult males of *C. villosum* (N=12) and *A. watsoni* (N=7). We kept individuals for a maximum of five nights in a 4.4×4.5×2.1 m flight cage (framework of metal tubes, covered with regular mosquito netting) in the forest near the field station. Bats were fed with fresh fruits from *F. obtusifolia* or *F. nymphiifolia* collected directly from branches of the tree where the animals had been captured. We used only ripe fruits for the experiment that had been collected the same day and were not infested with parasites such as the larvae of parasitic fig wasps or moths, because

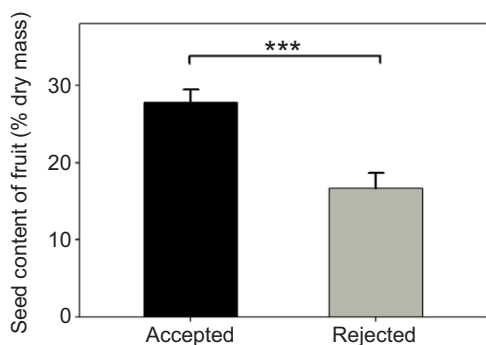


Fig. 4. Seed content of fruits from different fig species. Fruits are classified as ‘accepted’ or ‘rejected’ depending on the fruit-choice experiment and data from the literature. Values are means±1 s.e.m.; *** $P\leq 0.001$.

previous experiments (Korine and Kalko, 2005) had shown that infested figs were mostly rejected by the bats. No figs were collected from the ground. In the flight cage we presented whole fig fruits attached to a horizontal, free-hanging branch (supplementary material Fig. S1). We offered water in a 1×1 m pool, which seemed to be especially important for *C. villosum*. We observed five individuals drinking water during flight. All experiments were conducted with a single bat in the flight cage and started one night after capture, so that the animals had time to habituate.

Not all individuals could be used for every experiment and animals that refused to eat were released. After the experiments, bats were released at the place of capture, marked by a collar with an individual number (Handley et al., 1991). *Artibeus watsoni* were not marked this way because of known issues with this method in this species (E.K.V.K., personal observation); instead, they were marked by a wing punch with a sterile biopsy punch (Stiefel, Germany, diameter 4 mm). Recapture of marked bats during former studies had revealed that the hole in the wing membrane did not affect the animals' health or flight ability and would heal within 3–4 weeks (Wilmer et al., 1999; Kerth et al., 2000) but left a recognizable scar.

Processing time of figs and chewing movements/mastication time

To test whether seed-chewing *C. villosum* invested more time in the processing of figs compared with other fig-eating bats, we conducted feeding experiments with seven *C. villosum* and five *A. watsoni* individuals. The bats were offered similar-sized fresh *F. obtusifolia* (7–10 g) and *F. nymphiifolia* (6–8 g) fruits, placed on a branch in the flight cage. Individual fruits were weighed prior to the experiment (Mettler A&D HR300; accuracy ±0.1 mg). The feeding process was videorecorded with Sony DCR-HC39E and Sony DCR-SR70E camcorders set to Night-Shot mode. The cage was only slightly illuminated with infrared light (Hope and Bhatnagar, 1979; Winter et al., 2003).

Each individual was filmed during processing of five figs. The processing time (PT) started with the first bite the animal took of a fig and ended with the last pellet ejected by the bats. Breaks of inactivity in the feeding process longer than 10 s were subtracted from total PT.

Because of the large difference in body mass between *C. villosum* (22 g) and *A. watsoni* (12 g) and the slight differences in individual fig mass, we standardized processing time (PT_s) by dividing by the ratio between fig mass (M_f) and bat body mass (M_b):

$$PT_s = \frac{PT}{(M_f/M_b)}$$

To assess mastication time of pulp or seeds, we analysed five chewing processes for every individual per fig-eating process. In the video sequences, we measured the time from the moment the bat took a bite from a fig and started chewing until the ejection of the pulp pellet, and the time from the start of seed chewing until ejection of a seed pellet. We also counted chewing movements during pulp and seed chewing.

Seed predation and nutrient gain

We used *F. obtusifolia* in the feeding experiments for assessing the number of viable seeds in ejected pellets and faeces from *A. watsoni* and *C. villosum*, and for analysing nutrient gain from the fruits. The floor of the flight cage was covered with a clean plastic sheet from which we collected all the pulp and seed pellets ejected and the faeces produced during processing of at least 10 figs per bat. Pulp pellets from both species were examined under a stereo microscope and damaged seeds were counted and removed. Faeces of both species without seeds, and pulp pellets as well as chewed seed pellets from *C. villosum* were then weighed with an analytical balance (Mettler A&D HR300; accuracy ±0.1 mg) and dried in a forced-air drying oven (Precision Quincy Corporation, USA) at 60°C for 3 days. Afterwards, the samples were re-weighed to determine dry mass and the original water content was calculated. We were unable to collect faeces from *A. watsoni* because they almost exclusively consisted of seeds and fluid matter and did not contain enough seed-free material.

To determine the amount of nutrients extracted from the fruits by the bats, we compared the combined total nutrient content of ejecta, pulp, chewed seeds and faeces coming from a single fig with that of entire figs from the

same tree. For this, we weighed 10 fruits from each fig tree that we used for feeding the bats, then separated and dried seeds and fruit pulp. All samples were then ground to powder with a ceramic mortar and pestle. Samples were stored in air-tight vials at –20°C until shipment for analysis.

We quantified soluble sugar, soluble protein, lipids and nitrogen, following the methods outlined elsewhere (Bollen et al., 2004). Soluble sugar was analysed by measuring the equivalent of galactose after acid hydrolyzation of the 50% methanol extract (Kates, 1972). This corresponds to concentrations obtained with enzymatic analysis of glucose, fructose and galactose (Ganzhorn et al., 2004). To measure the concentration of soluble proteins (assumed to represent the fraction of proteins that can be extracted by animals easily), the plant material was extracted with 0.1 mol l⁻¹ NaOH at room temperature for 15 h and the protein content measured with the BioRad assay method. Nitrogen was determined with the Kjeldahl method. Nitrogen concentrations were not multiplied by the standard 6.25 conversion factor to get an estimate of crude protein as the conversion factor varies between plant parts (Milton and Dintzis, 1981) and the biological relevance of crude protein is still unclear (Rothman et al., 2012; Wallis et al., 2012). Lipids were quantified with the Soxhlet method (Sukhija and Palmquist, 1988).

Seed content and fruit-choice experiment

For every fig species used by the bats, we sampled at least four individual trees and analysed 10 fruits per tree (*F. obtusifolia*, *F. nymphiifolia*, *F. popenoi*, *F. citrifolia*, *F. insipida* and *F. maxima*). To determine the pulp to seed ratio, we separated every fig into seed and pulp parts and calculated the ratio based on dry mass. To assess mass per seed, we weighed 10 dry seeds from every fig separately.

For the fruit-choice experiment, the bats were presented with five *F. obtusifolia* fruits and five fruits from another fig species. The fruits were presented on opposite sides of a free-hanging, 200 cm long branch, at least 30 cm away from each other (supplementary material Fig. S1). The positions of the fruit species were randomly changed after every fruit-choice experiment.

The experiment started after the bats had already eaten two or three *F. obtusifolia* fruits to ensure their motivation. Because *C. villosum* were eating between five and 10 figs per night, we presented five *F. obtusifolia* fruits and five of the test *Ficus* species. In the case of the extremely small (1 g) *F. citrifolia*, we offered 20 fruits. As fruits from different fig species were not always available simultaneously in sufficient quantities, we could only do a limited number of behavioural experiments.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

All authors contributed to the study design, to the methods and to the writing of the paper. The fieldwork and data analysis were conducted by I.W.

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Supplementary material

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