

# Gut passage and secondary metabolites alter the source of post-dispersal predation for bird-dispersed chili seeds

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**Abstract** Plants can influence the source and severity of seed predation through various mechanisms; the use of secondary metabolites for chemical defense, for example, is well documented. Gut passage by frugivores can also reduce mortality of animal-dispersed seeds, although this mechanism has gained far less attention than secondary metabolites. Apart from influencing the severity of seed predation, gut passage may also influence the source of seed predation. In Bolivia, we compared impacts of these two mechanisms, gut passage and secondary metabolites, on the source of seed predation in *Capsicum chacoense*, a wild chili species that is polymorphic for pungency (individual plants either produce fruits and seeds containing or lacking capsaicinoids). Using physical enclosures, we isolated seed removal by insects, mammals, and birds; seeds in the trials were from either pungent or non-pungent fruits and were either passed or not passed by seed-dispersing

birds. Pungency had little influence on total short-term seed removal by animals, although prior work on this species indicates that capsaicin reduces mortality caused by fungi at longer time scales. Gut passage strongly reduced removal by insects, altering the relative impact of the three predator types. The weak impact of pungency on short-term predation contrasts with previous studies, highlighting the context dependence of secondary metabolites. The strong impact of gut passage demonstrates that this mechanism alone can influence which seed predators consume seeds, and that impacts of gut passage can be larger than those of secondary metabolites, which are more commonly acknowledged as a defense mechanism.

**Keywords** Frugivory · Granivory · Chemical defense · Mutualism · Seed fate

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## Introduction

Seed predators have profound impacts on plant population dynamics, species distributions, and diversity (Crawley 1992; Maron and Crone 2006; Bagchi et al. 2014), but the pattern and intensity of seed mortality due to predators are the result of a complex set of relationships between plant, animal, and microbial communities (Howe and Smallwood 1982; Nathan and Muller-Landau 2000; Fricke et al. 2014). Plants reduce seed mortality through a large variety of mechanisms (Janzen 1971). Often studied are chemical defenses, in the form of plant secondary metabolites, that can reduce overall antagonistic interactions and alter the composition of the natural enemy assemblage (Levin 1976; Howe and Jander 2008; Mithöfer and Boland 2012).

Changes to seed condition caused by frugivore seed handling and gut passage may, like secondary metabolites,

influence the source of seed mortality. However, the vast majority of studies examining gut passage concentrate on its impacts on germination through scarification or removal of germination inhibitors (Traveset et al. 2007; Samuel and Levey 2005), overlooking the potential impacts of changes to seed condition that could also influence interactions with antagonists. Observational and experimental studies that have compared survival of seeds with or without pulp removal have shown that pulp removal causes greater seed survival (Meyer and Witmer 1998; Lambert 2001; Fedriani et al. 2012) or mixed positive and negative impacts (Fedriani and Delibes 2013). These studies suggest that the observed impacts on seed survival are due to mechanisms unrelated to physical changes to seeds. Other experimental field studies confirm that changes to seed condition caused by gut passage can reduce interactions with antagonistic species (Fricke et al. 2013; Noss and Levey 2014). The relative impact of these two mechanisms—secondary metabolites and gut passage by frugivores—on the source of seed predation is untested.

We quantify the relative influence of gut passage and secondary metabolites on the source of post-dispersal seed removal of a wild chili (*Capsicum chacoense*) in Bolivia. Individuals of this bird-dispersed species either produce fruits containing or lacking capsaicinoids, the secondary metabolites that cause the pungency of chilies. Capsaicinoids are present in the fruit and on the seed coat of pungent chilies (Tewksbury et al. 2006). We used physical enclosures to isolate the impacts of three broad types of natural enemies (insects, mammals, and granivorous birds) on short-term seed removal in the field. By using an experimental design that crossed these treatments with pungency (pungent vs. non-pungent) and gut passage (gut-passed vs. not gut-passed), we were able to determine the independent and interactive impacts of pungency and gut treatment on seed removal by each type of seed predator. Namely, we were interested in determining whether gut passage, by removing capsaicinoids from pungent chili seeds, changes the ability of secondary metabolites to influence the source of seed predation.

## Materials and methods

### Species and study site

*Capsicum chacoense* is distributed in the Gran Chaco region of eastern Bolivia, western Paraguay, and northern Argentina. Adult plants are 0.3–1.5 m in height, have fruits that average 1 cm in length and contain an average of 18 seeds, and produce between five and 500 fruits each season between March and May (Tewksbury et al. 2008a). Birds disperse the seeds; short-billed elaenias (*Elaenia*

*parvirostris*) are the primary disperser at our study sites in Bolivia (Levey et al. 2006). Germination occurs between November and January after a dry season. We performed field experiments during the 2004 fruiting season at two study sites in southeast Bolivia, Rancho San Julian and Rancho Tres Aguadas.

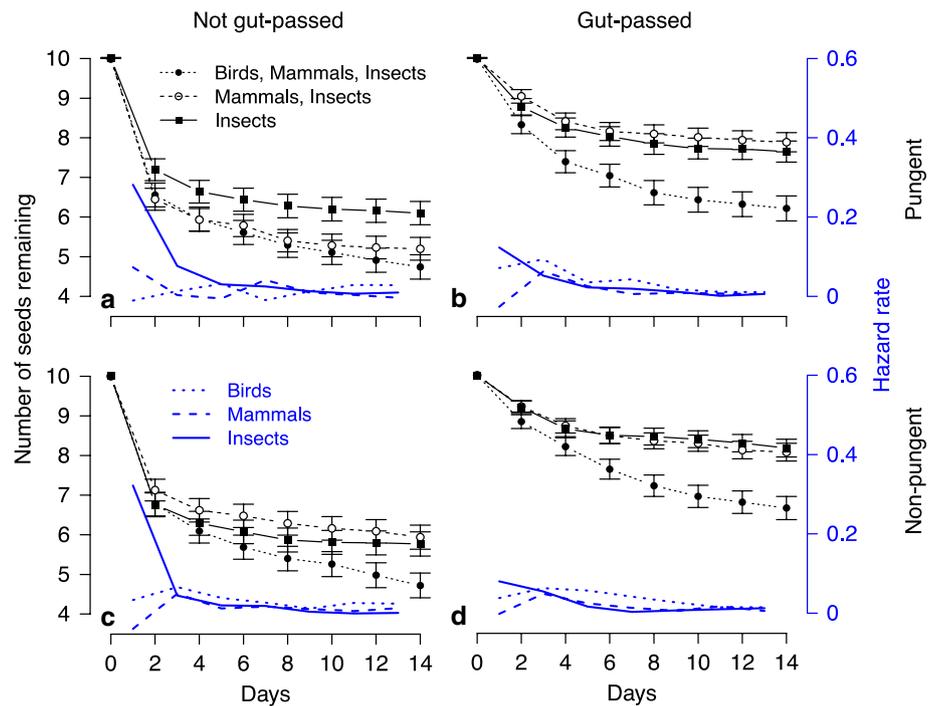
Post-dispersal seed predators include insects, mammals, and birds. During direct observations of seeds, we never saw insects other than ants removing seeds. Because ants often consumed seeds in place, and because we looked for and did not find viable seeds in refuse piles at ant colonies, we consider seed removal by ants to be predation. We did not observe seed consumption by granivorous mammals or birds and we know little about their dietary preferences at our sites, although granivorous vertebrates commonly occur there. Because chili seeds are small and thus unlikely to be cached (Vander Wall 2003), we assume seed removal by birds and mammals constitutes seed predation, and use the terms interchangeably.

### Field experiments

We established 75 plots along transects at each of the two study sites. At 12 locations within each plot, piles of ten seeds were placed on the ground. The number of seeds remaining was recorded every 48 h for 14 days. Two types of treatment (seed treatments and enclosure treatments) were used to determine how gut passage and secondary metabolites impact the source of post-dispersal removal. All seeds in a given pile were either non-pungent or pungent and were either consumed and defecated by captive *E. parvirostris* or were removed directly from fruit (Fricke et al. 2013). Seeds used for each combination of the seed treatments were pooled and mixed before being deployed. For the enclosure treatments, different types of enclosures were used to isolate the impact of three types of granivores: insects, mammals, and birds (Orrock et al. 2003). Each pile of seeds was covered with either 1.3 × 1.3-cm wire mesh that allowed insect access only, hexagonal wire mesh with ~4-cm<sup>2</sup> openings that allowed insect and mammal but not bird access, or was left uncovered to allow access by all granivore types. A factorial design dictated 12 combinations of variables: three enclosure types (birds and mammals excluded, birds excluded, no exclusion) × 2 gut treatment types (passed and non-passed) × 2 pungency types (pungent and non-pungent). One of these combinations was applied to all seeds in a given pile and the 12 piles in a plot received different treatments.

We analyzed seed survival to 2 weeks using generalized linear mixed-effects models with binomial distribution in the R package lme4 (Bates et al. 2015). Fixed effects were seed condition (gut-passed or not gut-passed), pungency (pungent or non-pungent), and

**Fig. 1** The number of seeds remaining and hazard rates over time in *Capsicum chacoense* predator exclusion experiments. *Lines with symbols (black)* represent the total number of seeds remaining within physical enclosures that allow access of distinct groups of seed predators (birds, mammals, and insects together; mammals and insects together; or insects alone). *Lines without symbols (blue)* represent the mean hazard rate (portion of seeds present at the previous time step that have been removed) attributable to birds, mammals, or insects individually. Seeds were pungent (**a, b**) or non-pungent (**c, d**) and were either taken directly from fruit (**a, c**) or were gut-passed by avian frugivores (**b, d**) (color figure online)



exclosure type; unique plot identity, nested within site, was used as a random effect.

For data visualization, we calculated removal attributable to each type of consumer. Removal by mammals and birds was calculated as the difference in seeds remaining between treatments in which the consumer type was or was not allowed access. For example, mammalian removal was the difference in survival between piles covered by the larger mesh treatment, which allowed access by insects and mammals, and the smaller mesh treatment, which allowed access by insects alone. Negative values are possible if there was higher removal under treatments excluding more consumer types than under treatments excluding fewer consumer types; such instances could be caused by low or no removal by one consumer type and natural variation in mean removal rate under each treatment. To assess if either pungency or gut passage caused a temporal difference in the source of removal, we report a hazard rate for each of the three remover types, indicating the removal rate between time steps when data were recorded. The hazard rate gives the portion of seeds that were removed out of the seeds that were present at the previous time step. In all figures we present data from both sites together.

## Results

Insects accounted for most seed removal across all combinations of gut passage and pungency seed treatments. By

the end of the study period, insects had removed 31 % of seeds. Granivorous birds removed 12 % of seeds across all seed treatments and mammals removed only 1.4 % of seeds. Gut passage greatly reduced overall removal when no predators were excluded (36 % of gut-passed seeds were removed compared to 53 % of not gut-passed seeds), whereas pungency had no overall impact on the portion of seeds removed (45 % and 43 % of pungent and non-pungent seeds were removed, respectively; Fig. 1).

The source of removal of *Capsicum chacoense* seeds strongly depended on both seed pungency and gut passage; the best-fit model had significant interactions between enclosure type, pungency, and gut passage (Table 1). Gut passage reduced seed removal by insects (Fig. 2), but removal by both mammals and birds did not differ between gut-passed and not gut-passed seeds. Pungent and non-pungent seeds generally experienced similar removal within each enclosure by gut-passage treatment combination (Fig. 2). However, not gut-passed pungent seeds experienced somewhat higher removal by mammals and slightly lower removal by birds compared to both not gut-passed non-pungent seeds and all gut-passed seeds.

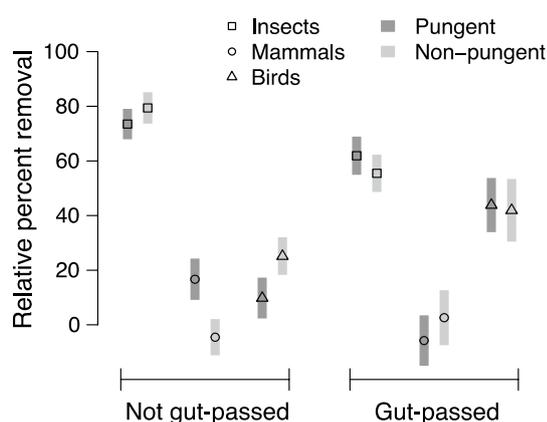
Temporal differences in removal rate existed only for insect removal of gut-passed versus not gut-passed seeds (Fig. 1). Insect hazard rates were high only during the first 2 days of the experiment for not gut-passed seeds. Otherwise, hazard rates for each remover type were similar over time across each gut-passage by pungency treatment combination.

**Table 1** Summary of models describing survival of *Capsicum chacoense* seeds under three treatment types: gut passage (GP) indicates whether seeds were taken directly from fruit or consumed by avian frugivores; enclosure treatments (EX) isolated impacts of three distinct predator types; pungency (PN) indicates whether seeds were taken from fruits that did or did not contain capsaicinoids

Model description	AIC	$\Delta$ AIC	P
GP + EX + PN + GP:PN + GP:EX + GP:EX:PN	13010	–	<0.0001
GP + EX + PN + GP:EX	13030	21	<0.0001
GP + EX + PN	13059	50	0.0001
GP + EX	13072	63	<0.0001
GP	13385	376	<0.0001
EX	13968	958	<0.0001
PN	14250	1240	0.0002
Intercept only	14262	1252	

Interaction terms are indicated by *colons*. Likelihood ratio tests were used to test if models improved the fit from the nested model described on the subsequent line or, for single-parameter models, from the intercept-only model

AIC Akaike information criterion



**Fig. 2** Contribution of insects, mammals, and birds to removal by the end of the study period of pungent or non-pungent *C. chacoense* seeds taken directly from fruit (*Not gut-passed*) or gut-passed by frugivorous birds (*Gut-passed*). The relative percent removal metric indicates, of the removal that occurred for each of the four pungency-by-gut passage combinations, what percentage is attributable to each predator type; within these combinations the percentages sum to 100 %. Note that mortality caused by birds and mammals was calculated as the difference between enclosure treatments, which allowed for removal values less than 0. *Data points* represent mean values using data from both sites. *Error bars* indicate  $\pm 1$  SE

## Discussion

We found strong impacts of gut-passage by frugivores on post-dispersal fate of *Capsicum chacoense* seeds, but comparatively small impacts of an ecologically important class of secondary metabolites (capsaicinoids). Gut passage halved seed removal by ants, which were the most common

type of seed predator. Relative to non-pungent seeds lacking capsaicinoids, pungent seeds experienced small reductions or increases in removal that were inconsistent across the gut-passage treatments. Although secondary metabolites are broadly recognized to modify interactions between plants and their antagonists (Rosenthal and Berenbaum 1992), our findings show that changes to seed condition caused by frugivore gut passage can likewise achieve targeted reductions in mortality at the seed stage. The influence of capsaicinoids on short-term post-dispersal predation differs from their role in another *Capsicum* species (Tewksbury and Nabhan 2001), showing that the effects on species interactions of individual secondary metabolites in ripe fruit can differ greatly among species and locations.

Our results show that frugivores can influence interactions between plants and seed predators through a broader set of mechanisms than commonly appreciated. Frugivores are often thought to provide benefits associated with movement away from parent plants, to uncolonized areas, and to favorable microsites (Nathan and Muller-Landau 2000). They can also provide benefits by changing the physical properties of seeds to facilitate germination (Traveset et al. 2007). The latter type of change can also influence seed fate and species interactions. For example, pulp removal by frugivores that do not or cannot disperse seeds can increase seed survival relative to seeds that remain embedded in pulp (Meyer and Witmer 1998; Lambert 2001; Fedriani et al. 2012). In our study system, gut passage by frugivores increases seed survival by reducing antagonistic interactions (Fricke et al. 2013). By isolating the impacts of three types of post-dispersal seed predators in this study, we show that gut-passage increases survival through reductions in specific sources of mortality (in particular, removal by ants), rather than through broad reductions across predator types.

Pungency influenced the relative importance of seed removal by ants, mammals, and birds, but capsaicinoids did not decrease seed removal by mammals. Indeed, our experiments show that mammals account for a very small portion of seed removal for *C. chacoense*. Other experiments indicate that context is critically important for the influence of capsaicinoids on predation. In previous work, Tewksbury and Nabhan (2001) showed that small mammals that live with pungent chilies in Arizona readily eat non-pungent chili fruits, but stop consuming chilies after consuming a pungent chili. Capsaicinoids thus protect seeds within fruits from small mammals that eat fruits from trees and shrubs. The negligible post-dispersal predation pressure by mammals we found at our study sites minimizes the ecological relevance of—or our ability to detect—directed deterrence of mammals by capsaicinoids in *C. chacoense*. Still, pungency did weakly alter the relative impact of the three types of seed predators that we excluded. Effects of capsaicinoids

on post-dispersal interactions may be more important for reducing mortality by other types of antagonists or over longer periods of time. For example, pungent seeds of this species have lower fungal pathogen loads before dispersal than do non-pungent seeds (Tewksbury et al. 2008b), which may lead to differences in post-dispersal mortality caused by fungal pathogens in the field.

Impacts of secondary metabolites and gut passage may interact if handling or gut passage by frugivores removes defensive chemicals; this could cause gut passage by frugivores to reduce seed survival (Noss and Levey 2014). In pungent chilies, which have capsaicinoids on the seed coat, gut passage could remove capsaicinoids and thereby prevent them from mediating subsequent interactions. If so, gut-passed pungent seeds should experience similar patterns of seed removal as non-pungent seeds. Our experiments show some support for this expectation because the relative impact of the three types of seed predators appears similar between gut-passed pungent seeds and all treatments involving non-pungent seeds. Still, the relative importance of the seed predator types is most similar among gut-passed seeds, whether from pungent or non-pungent plants. Perhaps the strong effect of gut passage on predation masks any influence pungency has on the relative impact of the different types of seed predators.

Gut passage caused temporal differences in the relative importance of predator types. Gut-passed seeds had much lower rates of predation by ants in the first 2 days of the experiment than did non-gut-passed seeds. By the end of the experiment, ants accounted for a smaller portion of the seed predation that occurred, and there was less overall predation for gut-passed seeds than for non-gut-passed seeds. As a result, birds also accounted for a larger portion of the removal among gut-passed seeds. Gut passage appears to remove volatile chemicals from *C. chacoense* seeds, including chemicals known to attract ants (Fricke et al. 2013). Our findings that gut passage reduces predation by ants, and that this targeted reduction in predation is short term, shows that this “chemical camouflage” benefit of frugivory can indeed target a specific predator type. Capsaicinoids could potentially strengthen this benefit because they increase gut retention times (Tewksbury et al. 2008a), and could thus more thoroughly remove attractants. However, pungent seeds did not have larger reductions in insect predation compared to non-pungent seeds.

Our comparison of two mechanisms by which plants can avoid seed predation showed that an underappreciated mechanism, gut passage by frugivores, is a much stronger influence on the source of post-dispersal seed predation than secondary metabolites, at least in our study system. Contributing to recent findings regarding the non-spatial impacts of frugivores (Fricke et al. 2013; Fedriani and Delibes 2013), this work documents how a mutualistic

interaction—frugivory—can indirectly influence the source and severity of subsequent antagonistic interactions. Frugivores thus alter interactions with antagonists through spatial and non-spatial mechanisms.

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**Author contribution statement** D. L., J. T., and D. H. designed the experiments. J. T. and D. H. conducted the field experiments. E. F. performed the statistical analyses and wrote the first draft of the manuscript. All authors revised the manuscript.

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