Evolutionary ecology of pungency in wild chilies


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The primary function of fruit is to attract animals that disperse viable seeds, but the nutritional rewards that attract beneficial consumers also attract consumers that kill seeds instead of dispersing them. Many of these unwanted consumers are microbes, and microbial defense is commonly invoked to explain the bitter, distasteful, occasionally toxic chemicals found in many ripe fruits.

This explanation has been criticized, however, due to a lack of evidence that microbial consumers influence fruit chemistry in wild populations. In the present study, we use wild chilies to show that chemical defense of ripe fruit reflects variation in the risk of microbial attack. Capsaicinoids are the chemicals responsible for the well-known pungency of chili fruits. Capsicum chacoense is naturally polymorphic for the production of capsaicinoids and displays geographic variation in the proportion of individual plants in a population that produce capsaicinoids. We show that this variation is directly linked to variation in the damage caused by a fungal pathogen of chili seeds. We find that Fusarium fungus is the primary cause of predispersal seed mortality, and we experimentally demonstrate that capsaicinoids protect chili seeds from Fusarium. Further, foraging by hemipteran insects facilitates the entry of Fusarium into fruits, and we show that variation in hemipteran foraging pressure among chili populations predicts the proportion of plants in a population producing capsaicinoids.

These results suggest that the pungency in chilies may be an adaptive response to selection by a microbial pathogen, supporting the influence of microbial consumers on fruit chemistry.

directed deterrence | frugivory | fruit chemistry | secondary metabolite | Capsicum chacoense

The evolution of fruit, a reward for animal dispersal of seeds, is a commonly cited example of a key innovation in the radiation of angiosperms (1–3). However, the nutritional qualities of fruit pulp that are responsible for attracting beneficial dispersers also attract consumers that are detrimental to plant fitness. These consumers range from vertebrate and invertebrate seed predators to microbial consumers of fruits and seeds that reduce the likelihood of dispersal and the viability of seeds (4).

Fruit chemistry is commonly thought to mediate these interactions, either by deterring seed predators (4–6) or reducing microbial attack of fruits and seeds (4, 7, 8). These mechanisms are not mutually exclusive, but chemicals that deter fruit consumption often affect a wide range of species (7, 9), and defensive chemistry in ripe fruit must be sufficiently targeted toward detrimental organisms to allow consumption by vertebrate seed dispersers. Fruit secondary compounds that deter microbial consumers without reducing seed dispersal by vertebrates are thought to be far more plausible than secondary compounds that selectively deter vertebrate predators (7), because microbial fruit consumers are uniformly negative in their impacts on plant fitness (4) and are farther removed in their morphology, physiology, and mode of consumption from vertebrate seed dispersers than are other unwanted consumers (4, 7).

Microbial deterrence is thus a primary hypothesis explaining the presence of noxious, bitter, and sometimes toxic chemicals in many ripe fruits; the negative effects these chemicals often have on vertebrate dispersers are assumed to be balanced by the benefits of deterring microbial consumers. Unfortunately, this hypothesis remains largely untested, because no work to date has shown that variance in microbial pathogen pressure is related to variance in the chemistry of ripe fruits in wild populations.

A strong test would require a species in which fruit chemistry is well known, likely to protect against microbial pathogens, unique to the fruit, and highly variable. The most famous plants with these qualities are chilies (genus Capsicum). Chilies were one of the first plants domesticated in the New World (10), and they are now consumed by one in four humans daily (11), largely because of the pungency produced by capsaicinoids. Capsaicinoids are well characterized (9) and broadly antimicrobial (12–14). In fact, early humans likely selected chilies for use and domestication expressly because of their antimicrobial properties (12, 15). Finally, because capsaicinoids are found only in the fruit of Capsicum species and their concentrations increase during fruit ripening (16), the function of these chemicals is likely restricted in the fruit itself, not attributable to alternative functions in other parts of the plant (17).

Chilies thus provide an exceptionally clear window into the function of fruit chemistry, and our recent rediscovery of a polymorphism for capsaicinoid production in wild populations of multiple chili species (18) provides the variability we need to explicitly examine the function of these chemicals in wild populations. We have studied this polymorphism most intensively in Capsicum chacoense Hunz., which is native to the Chaco region of Bolivia, Argentina, and Paraguay (19). In polymorphic populations, C. chacoense plants producing fruits that contain capsaicinoids grow alongside plants with fruits that are nutritionally similar (20) but completely lack capsaicinoids (18) [see supporting information (SI)]. In addition, the proportion of plants producing capsaicinoids varies widely among populations. At the southwestern end of our 300-km-long study area in southeastern Bolivia, the polymorphism is virtually absent; most populations contain only pungent plants.

To the north and east of this area, nonpungent plants gradually increase in frequency, until >70% of individuals lack pungent traits, and the few plants that do produce pungent fruit have capsaicinoid concentrations barely one-third the level found in completely pungent populations (18).

We use this geographic gradient as a tool to study the impact of microbial pathogens on fruit chemistry, and we made the following predictions: (i) Microbial fruit pathogens will have a large negative impact on nonpungent chilies, (ii) capsaicinoids will reduce microbial damage to chili fruits and seeds, and (iii) among populations, the proportion of plants producing capsaicinoids will increase as the intensity of microbial attack increases.


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Impact of Microbial Pathogens. Across all populations in this system, the only significant cause of predispersal fruit and seed damage is microbial infection. This damage appears to be caused primarily by a single fungal species, *Fusarium semitectum* Berkeley and Ravenel (hereafter *Fusarium*). *Fusarium* infection of seeds causes discoloration that is easy to score, and we found *Fusarium* infection in \( >90\% \) of all ripe fruits sampled across our populations (\( n = 305 \) fruits). The vast majority (95\%) of these infections were provisionally attributable to *Fusarium*, which rots chili fruits and kills seeds. Even at low levels of infection, *Fusarium* causes substantial reductions in seed survival (Fig. 1A). Its entry into fruits is facilitated by hemipteran bugs that pierce the pericarp of fruits with their proboscises (Fig. 1B). This piercing introduces *Fusarium* into the fruit and seeds, leaving visible scars on the fruit surface, which turns black as the fungus invades (Fig. 1B and C). We randomly selected single ripe fruits from pungent and non-pungent plants in our primary study site (called San Julian), counted foraging scars on the fruit, and scored all seeds in each fruit for degree of *Fusarium* infection. Fungal infection of seeds increased with the number of foraging scars on the fruit (\( F_{1,67} = 8.0, P = 0.006; \) Fig. 1D), and seeds from fruits without signs of insect damage showed no signs of fungal infection (odds ratio 7.3, Cochran’s \( \chi^2 = 10.8, P = 0.001; \) Fig. 1D).

Capsaicinoids and Microbial Damage. The same data gathered to assess the impact of hemipteran foraging on fungal infection also suggest a strong antifungal role for capsaicinoids. Although fungal infection of seeds increased with the number of hemipteran-foraging scars in both nonpungent and pungent fruits, the slope of this relationship was significantly steeper in nonpungent fruits (\( F_{1,66} = 55.81, P < 0.0001; \) Fig. 1D). Thus, for a given level of hemipteran foraging pressure, seed infection rates in nonpungent fruits are almost twice as high as in pungent fruit (\( F_{1,67} = 12.4, P = 0.001; \) Fig. 1D). We experimentally verified this susceptibility of nonpungent fruits to *Fusarium* by placing cages over randomly selected pungent and nonpungent plants in the same polymorphic population such that birds were prevented from removing fruits, but *Fusarium*-transmitting hemipterans had free access. We let these fruits mature naturally, then removed and scored their seeds for...
**Fusarium** infection. Degree of infection was more than twice as high on seeds from nonpungent plants than on seeds from pungent plants ($F_{1,33} = 6.2, P = 0.018$; Fig. 2A).

Nonpungent and pungent fruits are visually indistinguishable in the field, and their nutritional profiles are virtually identical (20). Nonetheless, the large difference in seed infection we observed between pungent and nonpungent plants could be because of a factor other than the presence or absence of capsaicinoids. Fungal loads in pungent fruit were 45–55% lower than in nonpungent fruit (Figs. 1D and 2A). If this reduction were caused by the presence of capsaicinoids in the pungent fruit, we should be able to generate a similar effect size in a more controlled experimental setting where capsaicinoid content is the sole independent variable. To test this we created artificial fruit media that mimicked the nutritional composition of *C. chacoense* fruit (20), differing only in the presence and concentration of the two primary capsaicinoids, capsaicin and dihydrocapsaicin. Inoculating these media with *Fusarium* isolates cultured from *C. chacoense* seeds from the same population showed that both capsaicin and dihydrocapsaicin cause strong dose-dependent inhibition of *Fusarium* growth (Fig. 2B, quadratic fits, $r^2 > 0.9$). More importantly, at the capsaicinoid levels found in our focal population (Fig. 2B, arrows), capsaicin reduced *Fusarium* growth by 33%, and dihydrocapsaicin reduced *Fusarium* growth by 16%. Together, these chemicals fully account for the observed reduction in *Fusarium* seed infection in pungent fruit (predicted reduction based on capsaicinoid concentrations = 49%, the 95% CI of observed reduction = 41%–80%).

**Fruit Chemistry and Fungal Selection.** Capsaicinoids thus protect chili fruits and seeds from a fungal pathogen that severely reduces seed viability. If this process shapes the chemistry of chili fruits, changes in fungal selection pressure among chili populations should lead to parallel changes in the chemical defense of chili fruits, explaining among-population variation in capsaicinoid production. This prediction was supported. We surveyed seven chili populations, distributed across a 1,600 km² area in eastern Bolivia (Fig. 3A). In each population, we randomly marked on pungent and nonpungent plants in the same population and scored all seeds on both sides from 0 (no obvious infection) to 5 (highest level of infection) and summed each seed’s two scores, creating a single score from 0 to 10.

**Foraging Scars and Fungal Pressure.** We counted the number of hemipteran foraging scars on randomly selected ripe fruit, then removed all seeds and scored them for fungal infection. For analysis, we used an ANCOVA design with regressions fit through the origin (Fig. 1D).

**Pungent Versus Nonpungent Fungal Loads.** Randomly selected unripe fruits were marked on pungent and nonpungent plants in the same population and left on plants until they had fully ripened. Seeds were then removed and scored for fungal infection. We used a linear mixed model to compare fungal loads between pungent and nonpungent fruit, blocking on year, plant, and fruit within plant. Pungency was a fixed effect, and *Fusarium* infection score was the dependent variable.
**Fusarium and Seed Survival.** Seeds from pungent and nonpungent plants were stored in the field through the dormancy season (April to October). Germination trials were conducted in the field, using moist filter paper on natural soil. Seeds were scored for *Fusarium* infection as described. Seed survival (Ss) was assessed as Ss = g + (1 – g × v), for each level of *Fusarium* infection (0–10), where g is the percentage of ungerminated seeds, and v is the percentage of ungerminated seeds testing positive for metabolically active tissue with tetrazolium chloride (26). We used a total of 3414 seeds for these trials. See the SI for detailed methods and results.

**Artificial Fruit Media.** To mirror the nutrient profile of ripe C. chacoense fruits (20), we created 11 batches of artificial fruit media (see the SI for the recipe), adding one of five concentrations of capsaicin or dihydrocapsaicin (0.25, 0.5, 1, 2, and 4 mg/g) dissolved in methanol to 10 of the batches, and added an equal amount of methanol as a control to the 11th batch. We then poured sterile media into 12-well plates (n = 24 plates, 288 wells) and inoculated media in the center with a small plug of *Fusarium* taken from one of four isolates, replicating each isolate in each treatment six times. Radial mycelial growth was measured at 72 h, and growth on treatment media was standardized relative to growth on control media, lacking capsaicinoids.

Complete Methods and associated references are available in the online version of this article.

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