Supporting Information

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SI Text

This section contains supporting information (SI) *Methods and Results*, Table S1, and Fig. S1. Table S1 provides detailed results for effects of *Fusarium* infection on seed germination and viability. Fig. S1 provides the frequency distribution of fungal infection in our primary study site.

SI Methods

Study Sites. Our primary study site was Rancho San Julian, Santa Cruz, Bolivia (19.77°S × 62.70°W). All field experiments were conducted using a dense population of chilies protected from grazing. In this population, 47% of plants bear only pungent fruit and 53% bear only nonpungent fruit (n = 120 plants). We used this site as well as two other sites—Yukeriti (54% pungent; 20.263°S × 63.261°W) and Tres Aguadas (100% pungent; 21.520°S × 63.781°W)—to determine the relationship between the number of hemipteran foraging scars and fungal infection.

Pungency. The two distinct chemical phenotypes (plants with fruit containing capsaicinoids and plants with fruits completely lacking in capsaicinoids) have been observed, and populations with both pungent and nonpungent individuals can be found throughout much of the lowland Bolivian Chaco (1). Capsaicinoids are completely absent in nonpungent phenotypes, whereas the concentration of capsaicinoids in pungent phenotypes forms a second continuous chemical trait, ranging from about 3 mg/g fruit dry mass to about 9 mg/g dry fruit mass (1). The changes in capsaicinoid concentration are under partial genetic control but have a large environmental component (2). Because nonpungent chilies contain no capsaicinoids (1), and plants containing capsaicinoids are obviously pungent to taste, with the least pungent populations containing fresh-fruit capsaicinoid levels that are more than 15 times higher than found in fresh Jalapeno peppers (3), field determination of pungent to nonpungent ratios in polymorphic populations is straightforward (1). Research in cultivated species has identified a single locus responsible for this polymorphism (4, 5). However, a recent study reporting a single nucleotide polymorphism (SNP) in multiple species failed to find a similar marker in *C. chacoense* (6). Research on the specific genetic mechanism underlying the polymorphism in this species is ongoing.

Fungal Infection of Seeds. Preliminary trials showed that a single fungal morphotype accounted for the vast majority of seed infection. To quantify this pattern, we categorized all fungal morphotypes found on 500 seeds collected from fruits in the field. The dominant morphotype (pictured in Fig. 1*A* above the abscissa) accounted for >95% of observed seed infection, and occurred on 77% of all sampled seeds. This morphotype was easily scored, as it spreads under the pericarp of the fruit as a black spot (Fig. 1*C*) and forms a black mat on the seed, which darkens and spreads during infection (Fig. 1*A*). We scored all seeds in the same way, using the same standard, from 0 (no fungus) to 5 (highest infection). We combined scores from the two sides of each seed to create a total score of infection from 0 to 10. In all cases, seed scoring was done blind, such that observers had no knowledge of pungency or treatment.

Fungal Identification. To identify this dominant fungal morphotype, we established fungal isolates in culture and used morphology and sequence data to identify the isolates. We isolated fungi by placing seeds on potato dextrose agar containing 0.5% ampicillin, tetracycline, and streptomycin. For DNA extraction, we transferred single-spore isolates to potato dextrose agar and let them grow for 5 days at 25°C to stimulate growth of aerial hyphae. We used a sterile scalpel and forceps to transfer fresh mycelia to lysing matrix tubes. We extracted DNA using the protocols and materials supplied in the FastDNA Kit and the FastPrep Instrument (Qbiogene), except that we eluted DNA using 100 μl Tris/EDTA (TE) buffer instead of DES. We amplified the ITS1–5.8s-ITS2 region of nuclear ribosomal RNA with ITS1 and ITS4 primers (7) using 10 μl reaction mixture consisting of 2 μl DNA, 1 μl Tris-HCl buffer, 0.8 μl 2.0 mM $MgCl_2$, and 0.2 μl each of 200 μM dNTPs, 0.5 units/μL TaqDNA polymerase, and 0.125 μM primers. We initiated PCRs with a 3 min dwell at 94°C, followed by 31 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C. The final extension was 5 min at 72°C. To confirm a single product, amplified DNA fragments were analyzed in a 1.5% agarose gel stained with ethidium bromide. We cleaned PCR products for direct sequencing by aliquoting 1.5 μl of shrimp alkaline phosphotase and 0.5 μl exonuclease into each 10 μl reaction and incubating at 37°C for 30 min and 80°C for 15 min. We sequenced the cleaned PCR products using ITS2, ITS3, and ITS4 primers (7). We cleaned the sequencing reactions using a salt-free ethanol precipitation and sequenced the samples on an ABI 3100 Automated Capillary DNA Sequencer (Applied Biosystems) by following the manufacturer's protocols.

We used BLAST (www.ncbi.nlm.nih.gov/blast/blast_program.shtml) to search the NCBI GenBank database for related sequences. We found ≥99% sequence similarity with *Fusarium semitectum* (synonyms = *F. incarnatum* and *F. pallidoroseum*), *F. chlamydosporum*, *F. chlamydosporum var. fuscum*, and *F. equiseti.* We then used morphological characteristics (8) of cultures grown on carnation leaf agar to identify the isolates as *Fusarium semitectum*. We consider this a conditional identification.

Scoring Seeds for Fungal Infection. In 2006 we randomly selected one fruit from each plant and counted hemipteran foraging scars on these fruits (n = 73 fruits; Fig. 1*D*). We then opened the fruit and scored all seeds within each fruit and calculated an average fungal infection score for all of the sides within a given fruit (Fig. 1*D*). Because we had a direct measure of hemipteran foraging pressure for each fruit, we used this to control for foraging pressure before examining differences in fungal infection between pungent and nonpungent plants. We used ANCOVA with regressions for pungent and nonpungent plants fit through the origin, because fruits without foraging scars lacked fungal infection (Fig. 1*D*).

We compared fungal infection of seeds from pungent and nonpungent plants at San Julian in two years (2003 and 2004). We used the same plants in both years. Each year we caged the plants to prevent birds from removing fruit. Randomly selected unripe fruits were marked on ten pungent and ten nonpungent plants, and all fruits were removed at the same time, 25 days after marking (when all fruits were mature). Seeds were scored after the first day of germination trials (see *Germination and Fungal Infection*). To compare levels of seed infection between pungent and nonpungent plants, we used linear mixed models, incorporating year, plant, and fruit within plant as random effects; pungency as a fixed effect and *Fusarium* infection score as the dependent variable.

Germination and Fungal Infection. We harvested fruits from ten pungent and ten nonpungent plants and removed the seeds by

hand (n = 2,091 seeds). We stored them at our primary field site in a ventilated box through the dry season (April–October), when seeds are typically dormant. Germination experiments, conducted in 2004, were started at the time of the first rains in November and December, when the majority of seeds naturally germinate. To germinate seeds, we placed seeds in cups of natural soil topped with moist filter paper and scored all seeds for *Fusarium* on the first day of the germination trial. We monitored germination for 6 weeks. To determine the effects of *Fusarium* on the viability of ungerminated seeds, we germinated a second set of seeds (n = 1,323 seeds collected from fruit from the same plants) using the same conditions and timing, and scored ungerminated seeds for *Fusarium* after 6 weeks. We then cut all seeds in half, soaked them overnight in a dilute (0.5%) tetrazolium solution, and examined the endosperm of each seed under a dissecting microscope. All seeds with intact endosperm that also stained positive for metabolically active tissue were considered viable (9).

We used general linear models with binomial error distribution to determine the effect of *Fusarium* infection and pungency on seed germination and the viability of ungerminated seed separately (Table S1). To characterize the overall effects of fungal attack on seed viability, we combined data on germination with data on viability of ungerminated seeds to calculate seed survival (Ss) as $g + (1 - g \times v)$, for each level of *Fusarium* infection (0–10), where g is the percent of seeds germinating after 6 weeks, and v is the percent of ungerminated seeds scored as viable. We then used nonlinear regression to characterize the relationship between seed survival and *Fusarium* infection.

Artificial Fruit Media. Artificial fruit media was made based on nutritional analysis of ripe *C. chacoense* fruits (10). We used 3.6 g fructose, 3.6 g glucose, 4.0 g soy protein, 11.5 ml safflower oil, 0.6 g NaCl, 14.0 g cellulose, 6.0 g agar, and 3 g lecithin in 500 ml H_2O. We used 12-well plates, placing all 11 treatments on each plate (0.25, 0.5, 1, 2, and 4 mg/g capsaicin, as well as 0.25, 0.5, 1, 2, or 4 mg/g dihydrocapsaicin, plus the control). We inoculated all media on a plate with 7 mm × 2 mm plugs of *Fusarium* taken from one of four isolates. We replicated this procedure six times per isolate in a randomized complete block design. All four isolates were taken from our primary study population, Rancho San Julian. We scanned plates every 12 h, and used the last scan in which none of the isolates had reached the edge of the plates (72 h).

To control for plate effects, we analyzed average linear growth of each treatment relative to the control treatment in the same plate. We used nonlinear regression to characterize the effects of capsaicin and dihydrocapsaicin concentrations on *Fusarium* growth, using mean relative growth for each treatment. Relative growth was calculated as a percentage of growth in the control treatment. Data were transformed as needed.

Pungency and Hemipteran Foraging Pressure. To determine if variance in fungal infection predicted the ratio of pungent to nonpungent plants, we surveyed a total of seven populations for pungency and hemipteran foraging pressure. Sites were selected along a 270 km northeast to southwest transect in southern Bolivia (Fig. 3*A*), based on previous surveys in this area (1). All sites were separated by a minimum of 15 km. At each site, we established a 40 × 50 m grid, and sampled all plants in this grid to determine hemipteran foraging pressure, and estimate the proportion of plants producing capsaicinoids. Two sites—San Julian and Agua Blanca (20.130°S × 62.870°W)—were in fenced corrals. We excluded these sites from our analysis, as all other sites were in habitat that had no signs of previous clearing or fencing, and we were concerned that ratios of pungent to nonpungent plants might be influenced by these actions. Inclusion of these sites does not change the relationship between hemipteran foraging pressures and the ratio of pungent to nonpungent plants: parameter values for nonlinear regression ($y = a\ln x + b$) without sites in corrals are a = 0.367, SE = 0.050; b = 0.102, SE = 0.070; r^2 = 0.91, $F_{1,5}$ = 39, P = 0.0008. Parameter values including these sites are a = 0.344, SE = 0.055; b = 0.112, SE = 0.075; r^2 = 0.85, $F_{1,7}$ = 35, P = 0.0004.

SI Results

The hemipteran community foraging on wild chilies.

We found a mixed community of hemipteran insects feeding on chilies after surveying over 1000 plants in seven study sites (locations shown in Fig. 3*A*). The insect community had many constant elements across locations, with most species and morphospecies being present at multiple sites. *Acroleucus coxalis* Stål (Lygaeidae) and three ubiquitous Pentatomidae accounted for 87.4% of all chili-feeding hemipteran records across sites.

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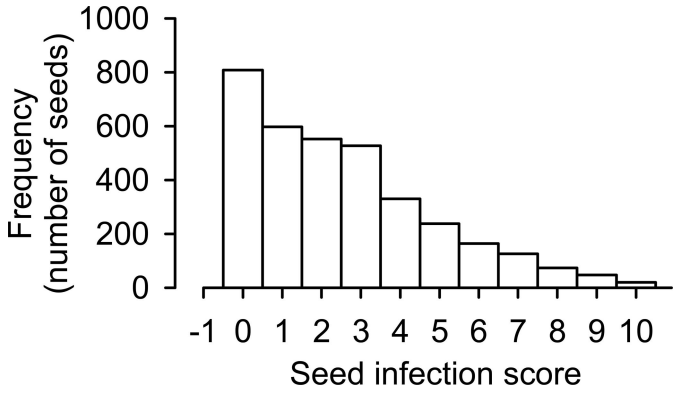

Fig. S1. Frequency distribution of seed infection scores used in germination and viability tests (n = 3,414). The median fungal score was 2.

Table S1. Binomial general linear model results for the effect of *Fusarium* infection and pungency on seed germination and the viability of ungerminated seed

	*b**	df	MS	*P*
Germination				
Pungency	0.22	1	19.00	0.02†
Fusarium	−0.77	1	51.33	0.0002†
Pungency × *Fusarium*	−0.30	1	6.68	0.15
Residual		121	3.41	
Viability of ungerminated seed				
Pungency ×	0.15	1	0.98	0.31†
Fusarium	−0.39	1	120.49	<0.0001†
Pungency × *Fusarium*	−0.13	1	14.14	0.0001†
Residual		1319	0.96	

*Intercept values for final models: germination = −1.91, viability = −0.24.
†Terms were retained in the model after model simplification (*F* tests used for germination due to overdispersion, and chi-square tests used for viability)