

WHERE DID THE CHILI GET ITS SPICE? BIOGEOGRAPHY OF CAPSAICINOID PRODUCTION IN ANCESTRAL WILD CHILI SPECIES

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Abstract—The biogeography of pungency in three species of wild chili in the chaco and surrounding highland habitats of southeastern Bolivia is described. We report that *Capsicum chacoense*, *C. baccatum*, and *C. eximium* are polymorphic for production of capsaicin and its analogs, such that completely pungent and completely nonpungent individuals co-occur in some populations. In *C. chacoense*, the density of plants and the proportion of pungent plants increased with elevation. Above 900 m, all individuals in all populations except two were pungent; nonpungent individuals in at least one of the two polymorphic populations were likely a result of spreading by humans. The occurrence of pungent and nonpungent individuals in three species of ancestral *Capsicum* and the geographic variation of pungency within species suggest that production of capsaicin and its analogs entails both costs and benefits, which shift from one locality to another. Determining the selection pressures behind such shifts is necessary to understand the evolution of pungency in chilies.

Key Words—Capsaicin, capsaicinoid, PSM, fruit secondary metabolite, fruit chemistry, intraspecific variation, biogeography, spice, *Capsicum chacoense*, *Capsicum baccatum*, *Capsicum eximium*.

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INTRODUCTION

Geographically structured phenotypic variation in plant morphology, physiology, and chemistry has provided a window into the evolution and coevolution of plant–animal interactions (Berenbaum and Feeny, 1981; Berenbaum and Zangerl, 1998; Schemske and Bradshaw, 1999; Thompson and Cunningham, 2002; Fornoni et al., 2004; Siepielski and Benkman, 2004). Such variation is often used to infer constraints on the course of evolution and to discern the relative importance of various traits in the diversification of clades (Futuyma and Mitter, 1996; Berenbaum and Zangerl, 1998). The explicit examination of intraspecific variation in plant chemistry has led to major advances in our understanding of plant–pollinator and plant–herbivore interactions (Lerdau et al., 1994; Adler et al., 1995; Cronin and Hay, 1996; Zangerl et al., 1997; Berenbaum and Zangerl, 1998; Baldwin, 1998; Schemske and Bradshaw, 1999; McDonald et al., 1999; Redman et al., 2001; Cipollini et al., 2002; Rowell and Blinn, 2003; Fornoni et al., 2004). In contrast, studies of intraspecific variation in the chemistry of ripe fruit are almost nonexistent (Izhaki et al., 2002; Tsahar et al., 2002; Tewksbury, 2002), despite the importance of secondary metabolites in fruit–frugivore interactions (Cipollini and Levey, 1997; Cipollini, 2000).

In some taxa, the defining feature of fruit is chemical, and it is in these taxa where variation may have the largest impacts on fruit–frugivore interactions. This is exemplified in *Capsicum*, the wild chilies. *Capsicum* contains 23–27 wild species and more than 2000 cultivars, derived from 3 to 5 of the wild species (Andrews, 1995; DeWitt and Bosland, 1996). Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and a series of homologous alkyl vanillylamides, called capsaicinoids, are responsible for the heat, or pungency, in chili pepper fruit (Iwai et al., 1979; Kawada et al., 1985; Govindarajan, 1986; Cordell and Araujo, 1993). Given that roughly a quarter of the world's population consume chilies each day (Cordell and Araujo, 1993), capsaicinoids are among the most widely used secondary metabolites in the world. To date, capsaicinoids have only been identified in the genus *Capsicum* (Govindarajan et al., 1987; Govindarajan and Sathyanarayana, 1991; Bosland, 1994). They are produced only in the fruit (Suzuki et al., 1980; Fujiwake et al., 1982) and are concentrated around the seeds and in the pericarp (Fujiwake et al., 1982; Suzuki and Iwai, 1984). Early taxonomies used the presence of capsaicinoids as a defining trait to characterize the genus *Capsicum* (Morton, 1938; Heiser and Smith, 1958), but not all *Capsicum* species produce capsaicinoids (D'Arcy and Eshbaugh, 1974).

Recent investigations support the hypothesis that capsaicinoid production is a monophyletic derived trait, as *Capsicum ciliatum*, a nonpungent species, appears basal to all pungent taxa (Walsh and Hoot, 2001), and pungency has been found in all taxa more derived than *C. ciliatum*. Furthermore, a single quantitative trait loci, *cap*, has been identified and accounts for 34–38% of the

phenotypic variation in capsaicinoid content (Blum et al., 2003). The only comprehensive theory advanced to explain the origin of pungency in *Capsicum* centers on an initial radiation from arid, high-elevation interior valleys of Bolivia (McLeod et al., 1982). The proposed ancestral species in this radiation was a form of *Capsicum chacoense*. More recent work using nuclear and chloroplast DNA supports a radiation of pungent taxa from drier mountainous regions, either in Bolivia or Peru (Walsh and Hoot, 2001), based on the earliest-branching pungent species (*Capsicum eximium* and *C. cardinasii*) and inconclusive bootstrap values separating the baccatum clade (*C. chacoense* and *C. baccatum*). Finally, the late discovery of a natural polymorphism for fruit pungency in an accession of *C. chacoense* from southeastern Bolivia (1959 collection by Paul Smith, USDA #PI260433, polymorphism discovered by P. Bosland, N.M. State) demonstrated that this species may not always have pungent fruit. Although early collectors have noted rare encounters with nonpungent plants (D'Arcy and Eshbaugh, 1974), details of this polymorphism have never been examined. The discovery and description of zones of polymorphism for capsaicinoid production may be a first step in understanding the origination and adaptive significance of capsaicinoids in wild fruit and their role in the radiation of the genus.

Our goal was to rediscover and map the occurrence of nonpungent *C. chacoense*, examine patterns of occurrence in light of natural and human-caused dispersal, and use this as a springboard to examine the biogeography of pungency in a broader taxonomic context. *C. chacoense*, *C. baccatum*, and *C. eximium* comprise three of the five most ancestral pungent *Capsicums* (Walsh and Hoot, 2001). All occur in southeastern Bolivia and northern Argentina, near to where the nonpungent form of *C. chacoense* was inadvertently collected in 1959. Our sampling and analysis focus on *C. chacoense*, and variation in capsaicinoid production across all three species in southeastern Bolivia, is examined.

METHODS AND MATERIALS

Field Sampling. We looked for *Capsicum* in the semiarid chaco and neighboring highland habitats in southwestern Bolivia (19°–22°S, 63°–65°W), sampling 29 natural populations of *C. chacoense*, 15 of *C. baccatum*, and 1 of *C. eximium* from February through April 2002–2005. These three species are easily distinguished by leaf and flower morphology, as well as plant stature. We examined all sampled populations for signs of obvious spatial patterning in the location and size structure of pungent and nonpungent plants that might be used to infer recent colonization of one form or another. Such patterns are described where present. In each population, ripe fruits were tasted from as many fruiting

TABLE 1. LOCALITY AND SAMPLING INFORMATION

ID	Location	Latitude	Longitude	Elevation (m)	Years ^a	n ^b	% P ^c	Density ^d
<i>C. chacoense</i>								
c1	Cerro, Colorado	-19.450	-62.372	396	2002	20	30	Low
c2	Ibasiriri	-19.564	-62.567	432	2002	56	55	Mid
c3	San Julian	-19.769	-62.700	490	2002-2004	41 (32-56)	29 (23-37)	Low
c4	Auga Blanca	-20.130	-62.870	533	2002	99	45	Mid
c5	Yuque	-20.263	-63.251	882	2003-2004	20.5 (20-21)	54 (35-71)	Mid
c6	Kurupaity	-19.628	-63.640	964	2002-2004	17 (10-30)	97 (90-100)	Mid
c7	Petrolero, lavertiente	-21.295	-63.300	371	2002, 2004	20.5 (16, 25)	81 (80-81.5)	Low
c8	Isiri	-21.346	-63.630	650	2003-2004	12.5 (8-17)	85 (75-94)	Low
c9	River ranch	-21.364	-63.635	664	2004	71	82	Mid
c10	Roadside 1	-21.395	-63.656	745	2004	21	100	Low
c11	Roadside 2	-21.445	-63.675	810	2004	17	100	Mid
c12	Roadside 3	-21.459	-63.694	949	2004	14	100	Mid
c13	Roadside 4	-21.456	-63.709	992	2004	16	100	Mid
c14	Palos, Blancos1	-21.43	-63.788	738	2002	130	100	High
c15	Palos, Blancos2	-21.446	-63.783	758	2003	70	100	High
c16	Tres Aguadas	-21.520	-63.781	855	2003-2004	100 per year	100	High
c17	Santa Ana	-21.554	-64.588	1925	2002	100	100	High
c18	Camino, Padkaya	-21.647	-64.623	1764	2002	45	100	High
c19	Calamuchita	-21.698	-64.624	1688	2002	232	62	High
c20	Roadside 5	-21.237	-63.579	502	2004	6		High
c21	Roadside 6	-21.348	-63.637	651	2004	8		High
c22	San Antonio	-21.289	-63.456	386	2005	2		High
c23	Capiatindi	-19.632	-62.608	451	2005	2		High
c24	Mariqui	-19.838	-62.931	583	2005	5		High

c25	Aguarati	-20.127	-63.119	700	2005	2	13 (6-25)	92 (88-100)	Low
c26	Yuque2	-20.290	-63.255	885	2005	1	10	60	Low
c27	Camatindi, Palavecino	-20.328	-63.280	813	2005	4	10	30	Low
c28	Cuevo	-20.448	-63.557	1096	2005	2	10	100	Low
c29	Ibibobo	-21.540	-62.992	343	2005	5			
<i>C. baccatum</i>									
b1	Kurupaity	-19.628	-63.640	964	2002-2004				
b2	Lagunillas	-19.622	-63.674	920	2005				
b3	Muyupampa	-19.883	-63.747	1222	2005				
b4	La Herradura	-19.500	-63.526	870	2005				
b5	Abapo chaco	-18.619	-63.269	594	2004		7		
b6	Gutierrez	-19.417	-63.530	978	2005		8		
b7	Roadside 5	-21.237	-63.579	502	2004		4		
b8	San Miguel	-20.070	-63.885	1016	2005		4		
b9	Monteagudo	-19.792	-63.993	1174	2005		3		
b10	Villa Montes	-21.268	-63.519	406	2005		1		
b11	Carapari	-21.826	-63.744	814	2005		3		
b12	Entre Rios	-21.517	-64.176	1234	2005		8		
b13	Cuevo	-20.448	-63.557	1096	2005		2		
b14	Yuque2	-20.290	-63.255	885	2005		1		
b15	North Yuque	-19.458	-63.240	828	2005		3		
<i>C. eximium</i>									
e1	Tarija	-21.417	-64.759	2027	2005		10	90	Low

^aYears sampled.

^bAverage number of plants sampled for pungency each year (range, if more than 1 yr).

^cAverage percent of plants pungent (range if multiple years). Percent pungent and plant density were calculated only for populations where >10 individuals were sampled.

^dDensity was estimated as follows: low is <10 plants per hectare; mid is 10-100 plants per hectare; high is >100 plants per hectare.

plants as possible to determine the ratio of pungent to nonpungent plants. This is an accurate method of capsaicinoid detection because humans can detect these compounds at <1.5 ppb (Scoville, 1912), a concentration far lower than is found in any pungent wild chili (see Results), and capsaicinoids are produced before fruit ripening. In field-testing plants for pungency, false positives are virtually impossible (if a plant lacks capsaicin, it will never taste pungent), but false negatives are possible if a particularly mild pungent plant is tasted after a more pungent plant. To guard against false negatives, multiple fruits from any plant that was determined to lack pungency were sampled, and we had multiple people check the pungency of the fruits. If anyone tasted capsaicin, the plant was considered pungent. In addition, some populations were sampled in multiple years to determine the stability of the ratios of pungent to nonpungent plants (Table 1). Voucher specimens were taken from each population and deposited at the Museo Historia Natural “Noell Kempff Mercado” and the Bolivian National Herbarium.

In one population (Figure 1, c3), permanent tags were affixed to all plants, and these were sampled in multiple years to verify that individual plants consistently produced either pungent or nonpungent fruit. In addition, seeds from pungent and nonpungent plants in five populations were collected and grown under identical conditions to quantify biogeographic variation in the concentrations of various capsaicinoids.

The density of individuals in all populations were estimated based on the area searched and the number of plants encountered. Because of differences in terrain and plant detectability among sites, all populations were classified into broad density classes. Low-density populations had <10 plants per hectare, medium-density populations had between 10 and 100 plants per hectare, and high-density populations had >100 plants per hectare. GPS locations of all populations were determined at the time of collection. In two populations (Table 1, c3 and c19), the location of all plants also was mapped, allowing us to examine fruit chemistry in a spatially explicit format.

Capsaicinoid Profiles of C. chacoense. To verify that nonpungent plants completely lacked capsaicinoids and to test for genotypic differences in capsaicinoid concentrations between populations of *C. chacoense*, seeds from five *C. chacoense* populations were collected and grown in a greenhouse to avoid environmental effects on capsaicinoid production (Harvell and Bosland, 1997). All plants were grown in 4-in. pots filled with uniform clay particles (Turface®, Profile Products LLC, Buffalo Grove, IL, USA) and kept moist through daily watering. The nutrient solution used was a standard 20–10–20 (NPK), rotated weekly with a 17–5–17 blend. Greenhouse temperatures averaged 23.4°C during the day and 17.4°C at night, under a 13:11 light/dark cycle. Fruit production started 90 days after germination. Fruits from pungent and nonpungent plants were analyzed from each population separately. Within

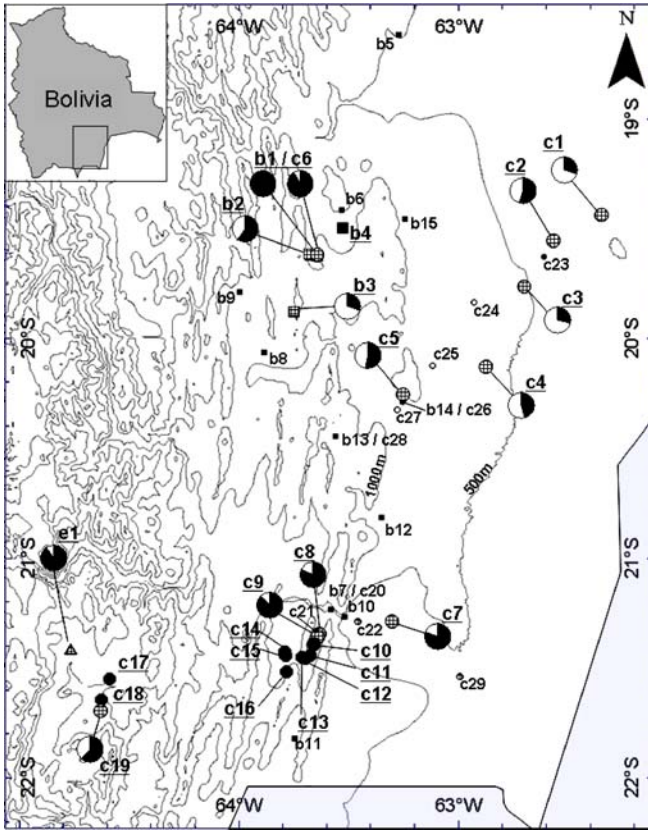


FIG. 1. Pungency distribution in sampled populations of *C. chacoense* (circles), *C. baccatum* (squares), and *C. eximium* (triangle) in southwestern Bolivia. Large symbols indicate populations where ≥ 10 plants were sampled for pungency; small symbols indicate the location of populations in which < 10 plants were sampled. Black symbols are completely pungent populations, hatched symbols are polymorphic populations, and white symbols indicate populations where no pungent plants were sampled. Pie charts indicate percent of plants pungent (black slice) in all polymorphic populations with ≥ 10 plants sampled. Contour lines represent 500-m elevation changes. Letter-number combinations refer to population data in Table 1.

each pungency by location combination, fruits from multiple plants with different maternal genotypes were bulk-sampled, creating a single, homogenous sample for analysis (10–30 genotypes used for each location by pungency combination). All fruit samples were harvested when fully mature, dried 48 hr at 65°C directly after harvest, and ground to a uniform particle size (skin, pulp, and seeds). This powder was then digested in 100% acetonitrile solution for 4 hr

at 85°C, following Collins et al. (1995). Total capsaicinoid concentrations (mg/g dry weight of the chili) and concentrations of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin were measured for each population using an Agilent 1100 high-performance liquid chromatography (HPLC), equipped with an autosampler and an Agilent XDS Zorbax C-18 Column. HPLC conditions and mobile phases followed established protocols for the analysis of individual capsaicinoids (Collins et al., 1995). Concentrations were derived by comparing peak area of samples to external standards of 1 mg/ml for capsaicin and dihydrocapsaicin (Sigma-Aldrich Co.).

Analysis. The relationship between the percent of pungent individuals in a population and elevation (log transformed) was examined using linear regression. Linear regression was also used to examine the effect of elevation on the concentration of total capsaicinoids, as well as the three individual capsaicinoids that were resolved. In this analysis, we corrected for multiple tests on the same data by using sequential Bonferroni adjustment, with a total of four tests (Rice, 1989). The relationship between population density and elevation was examined by using analysis of variance. In the one population where spatial structure was found in the location of nonpungent plants, we mapped the structure and tested for significance using a chi-squared test. Mapping of populations was performed using ArcGIS 9.0.

RESULTS

A total of 1481 *C. chacoense* plants in 28 populations, 93 *C. baccatum* plants in 15 populations, and 10 *C. eximium* plants in a single population (Table 1) were sampled. In all three species, populations composed of plants that produced only pungent fruit and other plants that produced only nonpungent fruits were found (Figure 1). These were termed polymorphic populations.

Biogeography and Habitat Associations of C. chacoense, C. baccatum, and C. eximium. The proportions of pungent plants in all populations in which 10 or more plants were sampled are reported, totaling 21 *C. chacoense* populations, 4 *C. baccatum* populations, and 1 *C. eximium* population. The smaller number of polymorphic *C. baccatum* and *C. eximium* populations prevent detailed analysis of patterns of polymorphism, but the biogeographies of all three species are described, detailing overlapping distributions, where appropriate.

C. chacoense populations were found primarily in xeromorphic chaco forest at lower elevations (Figure 1, c1–c5, c7), grading into montane dry forest in the inter-Andean valleys at higher elevations to the west (Figure 1, c14–c16), and extending to the transitional zones around Tarija (Figure 1, c17–c19). In the lower chaco forests, *C. chacoense* were found in stands dominated by *Schinopsis*, *Aspidosperma*, *Ziziphus*, and *Prosopis*. Other species producing

fleshy fruit concurrent with *C. chacoense* included *Celtis*, *Vallesia*, *Capparis*, and *Ravenna*. In localities where *C. chacoense* was found at intermediate elevations (Figure 1, c8–c16), the vegetation was similar to lowland chaco, with increasing moisture toward the west (Figure 1, c16–c18). Here, chilies were found in woodlands with extensive *Prosopis*, *Schinopsis*, and *Bromelia*. Between these two dry ecoregions, and along the eastern flank of the Andes, *C. baccatum* was found, located in moister habitat (Ribera et al., 1994). In the transition between the wet yungas and the dry chaco, *C. chacoense* and *C. baccatum* co-occurred (Figure 1, b1/c6, b14/c26, b13/c28, b7/c20). At higher elevations around Tarija, *C. chacoense* (Figure 1, c17–c19) and the lone population of *C. eximium* (e1) were found in a more open habitat characterized by *Prosopis nigra* and other leguminous trees. In these habitats, *Commiphora*, *Celtis*, *Cassia*, and *Capparis* were all seen fruiting at the same time as *Capsicum*.

Pungency, Plant Density, and Elevation. The proportion of pungent plants, the capsaicinoid content of pungent fruits, and the density of chili plants all increased with elevation for *C. chacoense* (Figure 2A and B). In all but two populations higher than 800 m, greater than 90% of the plants were pungent, creating a bounded association between proportion of plants pungent and elevation (Figure 2A). This relationship can be decomposed into populations below 800 m, where a significant increase in pungency with increased elevation was found (Figure 2; adj $r^2=0.53$, $P=0.01$; 10 populations < 800 m), and a zone of consistent high incidence of pungency above 800 m (Figure 2A).

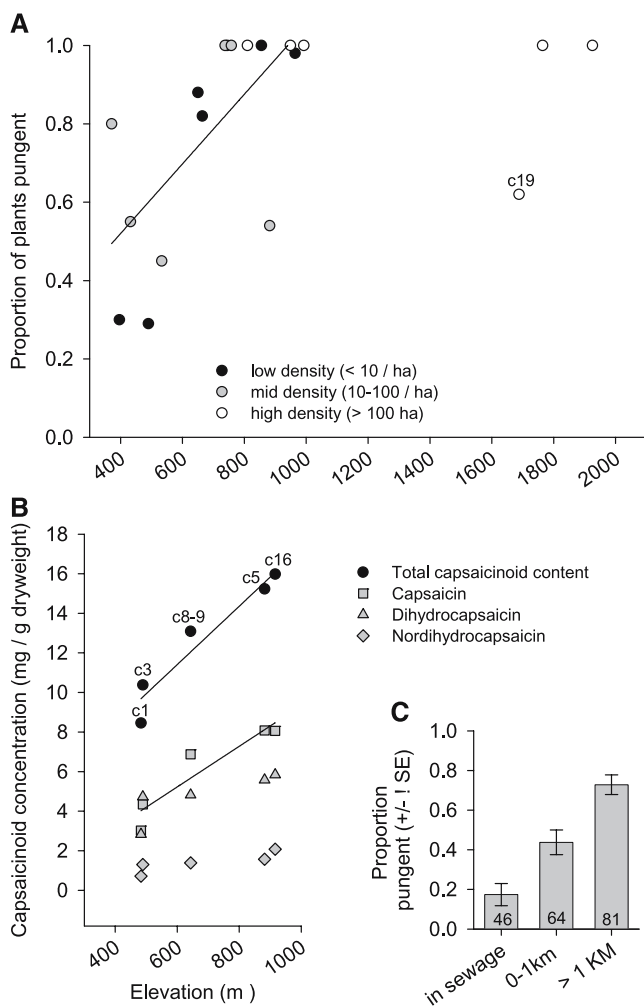
Population density also increased with elevation (Figure 2A; $F_{2,16}=6.8$, $P=0.007$); the mean elevation of low-density populations (<10 plants per hectare) was 530 m ($N=5$, $SE=73$ m), the mean elevation of mid-density populations (10–100 plants per hectare) was 778 m ($N=8$, $SE=75$ m), and high-density populations (>100 plants per hectare) averaged 1288 m in elevation ($N=6$, $SE=228$ m). Thus, compared to lower elevation populations, higher-elevation populations generally had more plants, and more of these were pungent.

In the five populations in which capsaicinoid production was analyzed, nonpungent plants were completely free of capsaicinoids, and total capsaicinoid content increased significantly with increasing elevation (adj $r^2=0.90$, $P=0.009$). All three capsaicinoids generally increased in higher-elevation populations (Figure 2B), with capsaicin showing the largest change with increasing elevation (adj $r^2=0.83$, $P=0.021$). This change was marginally significant after correction for multiple tests (Rice, 1989). Increases in dihydrocapsaicin and nordihydrocapsaicin with elevation (adj r^2 's=0.56 and 0.63, respectively) were not significant (P 's > 0.05).

In pungent plants, capsaicin was the most common of the capsaicinoids in four out of five populations (Figure 2B), averaging 6.07 ± 0.46 mg/g dry mass in

a ripe chili or $46.7 \pm 1.5\%$ of the total capsaicinoid content. Dihydrocapsaicin was the next most common, averaging 4.76 ± 0.24 mg/g dry mass or $37.8 \pm 0.9\%$ of the total capsaicinoid content, and nordihydrocapsaicin made up most of the remainder, averaging 1.4 ± 0.09 mg/g dry mass or $11.0 \pm 0.4\%$ of the total capsaicinoid content. In populations c3 where we sampled the same plants repeatedly over 4 yr, all plants consistently produced either pungent or nonpungent fruit ($N=25$ plants sampled across 3 yr, $N=15$ across 4 yr).

Dispersal by Humans. In one of the high-elevation polymorphic populations of *C. chacoense* (c19), the distribution of nonpungent plants appeared



highly clumped around two large (>1.4 m) nonpungent plants growing in a sewage outflow drainage for the town of Calamuchita. We sampled this population extensively to examine this pattern (232 plants sampled for pungency). Nonpungent plants in this population ($N = 88$) were concentrated around the two large plants (Figure 2c; $\chi^2_{2,189} = 35.1$, $P < 0.005$), and pungent plants became more and more dominant at increasing distance from these two large nonpungent plants (Figure 2c). Except for these two large individuals, all other nonpungent plants were small (<60 cm in height). This clumped pattern was not evident in other populations, and in the one low-elevation population in which we marked and mapped plants explicitly (c3, 123 plants), we found no clumping of nonpungent or pungent plants.

DISCUSSION

Genetic regulation of pungency occurs at a single locus, *C*, in *Capsicum annum* where accessions recessive for *c* fail to produce capsaicinoids (Blum et al., 2002). The gene required for production of capsaicinoids in cultivated chilies is *pun1* (Stewart et al., 2005). This understanding of the genetic control of pungency in *C. annum*, a highly derived species, may not apply to wild populations of *C. chacoense*, *C. baccatum*, and *C. eximium*, where our discovery of polymorphism for pungency demonstrates that pungency is not consistently present in more ancestral taxa of *Capsicum*. Of the five most ancestral wild species (Walsh and Hoot, 2001), *C. chacoense*, *C. baccatum*, and *C. eximium* are the only species with relatively wide ranges. *C. chacoense* is found throughout the chaco in Paraguay and Argentina (McLeod et al., 1982)

FIG. 2. (a) Proportion of plants in *C. chacoense* populations that are pungent, as a function of the elevation, for all populations with ≥ 10 plants sampled. Two high-elevation polymorphic populations are labeled. Linear fit is included for all populations <1200 m ($r^2 = 0.47$, $P = 0.0034$). (b) Concentration (mg/g dry mass) of total capsaicinoids (black circles), and the three individual capsaicinoids found in all three populations: capsaicin (gray squares), dihydrocapsaicin (gray triangles), and nordihydrocapsaicin (gray diamonds), as a function of the population elevation, for five populations grown from seed in identical greenhouse conditions (population labels indicated above black circles). Linear fit lines indicate significant relationships between elevation and the concentration of total capsaicinoids, and the primary capsaicinoid, capsaicin. (c) The distribution of nonpungent plants in relation to two large plants found in a sewage discharge drainage basin is shown for population c19. Bars are arcsine square-root-transformed percentage of plants pungent, error bars indicate one standard error of the mean, and number of plants sampled is indicated within the bars.

and as far west as Tarija and Sucre. According to McLeod et al. (1982, 1983), *C. chacoense* may be ancestral to *C. baccatum* and *C. eximium*. *C. baccatum* is also found in Paraguay and Argentina, but extends further north and west than *C. chacoense*—being found in Brazil and Peru as well (Eshbaugh, 1970), where it may have given rise to *C. tovarii*, a species endemic to southern Peru (Eshbaugh et al., 1983). Based on hybridization studies, *C. tovarii* is tightly linked to the *C. baccatum* complex (Tong and Bosland, 1999). *C. eximium* is a higher-elevation species, extending from the mountains in Argentina through the highlands in Bolivia, Peru, and Ecuador (Eshbaugh, 1976). *C. eximium* may be the progenitor of *C. cardinasii*, an endemic found only in the La Paz region in Bolivia with which it crosses readily (Eshbaugh, 1976; Walsh and Hoot, 2001).

If this interpretation is correct, the three most ancestral species of pungent chilies all have both pungent and nonpungent forms. Furthermore, while both *Capsicum cardenasii* and *C. tovarii* are reported as pungent, systematic sampling of these species has not occurred; they may be polymorphic, too. This interpretation fits well with McLeod et al. (1982) suggestion of a “nuclear area” of inter-Andean valleys northeast of Sucre, where divergent selection on ancestral *C. chacoense* led to both the purple-flowered group (*C. eximium*) and the white-flowered group (*C. baccatum*). According to this hypothesis, *C. chacoense* gave rise to *C. eximium*, which expanded into the dry highlands, and the white-flowered group (*C. baccatum*), which occupies semimoist lowlands. McLeod et al. further suggest that *C. baccatum*, through eventual migration down river to the Rio Grande, then into the moist tropics of the Amazon basin, radiated to form the moisture-loving *C. annum* group found in Brazil. This hypothesis relies on the importance of ecotones and heterogeneity in the physical environment in the creation of geographically explicit selection pressures (Stebbins and Major, 1965; Stebbins, 1972). Molecular data are currently insufficient to test this hypothesis (Walsh and Hoot, 2001), but the existence of polymorphic populations in *C. chacoense*, *C. baccatum*, and *C. eximium* refocuses attention to this area as a zone where early evolution of pungency may have occurred.

A more complete understanding of the early evolution of capsaicinoid production will require additional information on the link between nonpungent species, such as *C. ciliatum* (D’Arcy and Eshbaugh, 1974) and this clade of polymorphic species that appears to be ancestral to pungent *Capsicum* (Walsh and Hoot, 2001). Still, the finding that the first pungent *Capsicum* may all exhibit polymorphisms for capsaicinoid production has several implications for the ecology and evolution of these secondary metabolites.

The clinal variation in capsaicinoid production seen in *C. chacoense* and the existence of polymorphic populations of *C. baccatum* and *C. eximium* both suggest that capsaicin production is not uniformly beneficial. Furthermore, *C.*

chacoense populations are diffuse and widely scattered in the lowland chaco, and yet consistently exhibit polymorphisms for pungency, with at least 25% of the plants pungent in all populations in which at least 10 plants were sampled (Table 1). This pattern suggests either some form of frequency-dependent selection on pungency that prevents the pungent or nonpungent form from becoming fixed in the population, or some form of oscillating selection pressure, in which conditions favor pungent plants at some times and nonpungent plants at others, regardless of the frequency of the two phenotypes (Futuyma, 1998).

Teasing apart these possibilities will require a greater knowledge of the selective pressures acting on the entire fruit phenotype and the costs and benefits of secondary metabolite production (Berenbaum, 1995). Although the array of potential selective pressures on fruit secondary metabolites has been outlined (Cipollini and Levey, 1997; Cipollini, 2000), the full array of adaptive hypotheses has not been addressed in any system. The work that comes closest is that on emodin in *Rhamnus* fruit (Izhaki, 2002; Izhaki et al., 2002; Tsahar et al., 2002). This is the only system in which the effects of intraspecific variation in fruit secondary metabolites have been studied systematically. Variation in emodin concentrations among plants is correlated with nutrient concentrations (Izhaki et al., 2002). It affects both invertebrate and vertebrate fruit consumers (Tsahar et al., 2002, 2003) and shows intra- and interannual variations in its effect on consumers (Tsahar et al., 2002). These complex selective environments suggest that multiple adaptive functions may be the norm for secondary metabolite production (Schmitt et al., 1995; Cipollini and Levey, 1997), but a general theory of the pressures governing this variation has yet to appear and is unlikely until we place the adaptive benefits into a mechanistic framework that includes not only the benefits, but also the costs and potential trade-offs of secondary metabolite production (Berenbaum, 1995).

The discovery of three basil lineages all with polymorphisms for the production of a major secondary metabolite creates an unprecedented opportunity to examine both the costs and benefits of secondary metabolite production and to test these theories in three closely related taxa. Furthermore, in *C. chacoense*, nonpungent plants dominate populations in low-elevation chaco, and, among pungent plants, capsaicinoids (particularly capsaicin) occur at relatively low concentrations. As elevation increases, plant density increases, pungent plants dominate, and capsaicinoid concentrations become higher. These correlated patterns further suggest shifts in the relative costs and benefits of capsaicinoid production across elevation, and they may point to developmental or allocation trade-offs during fruit maturation. Because the pungent forms of all three of these species are harvested and consumed by locals, and *C. baccatum* (var. *baccatum*) has been domesticated (*C. baccatum* var. *pendulum*), human movement of genotypes might obscure evolutionary relationships. This applies equally to nonpungent forms of these species, as nonpungent plants are

indistinguishable from the pungent forms, except by tasting the fruit. We see possible evidence of the influence of humans in Calamuchita (c19) where nonpungent plants were concentrated around two large nonpungent individuals in the sewage outflow, suggesting that the large nonpungent plants may have arrived through human consumption of nonpungent chilies from elsewhere, followed by a gradual radiation from these two founders. This interpretation is speculative, but *Capsicum* are typically long-lived plants in the wild (Tewksbury et al., 1999), providing sufficient opportunity for establishment and subsequent local radiation to occur. More rigorous explanations will have to wait for information on relatedness and gene flow between populations. The existence of nonpungent forms at high elevations in two locations (c5 and c19) does suggest that whatever selective forces are responsible for the general shift from polymorphic to completely pungent populations with increasing elevation are not uniform: nonpungent plants can grow and reproduce in some high-elevation locations.

The clinal variation in pungency and percent of pungent individuals suggest either a lack of gene flow that prevents mixing of the pungent and nonpungent forms across this gradient, or a shift in selective pressures such that both pungent and nonpungent forms are maintained in the lowland chaco, but selection favors pungent plants at most high-elevation locations. We view the former explanation as unlikely for the entire gradient, given the clinal pattern across elevation and the fact that the distances between polymorphic and completely pungent populations are often small. For example, population c9, in which 18% of plants sampled lacked pungency, is less than 30 km from populations c14 and c15, in which 200 plants were sampled and all were pungent. Between these two populations, we found small populations of chilies that were totally pungent (Figure 1; populations c10–c13). The major dispersers of chili seeds in Bolivia are *Elaenia parvirostris* and *Turdus amaurochalinus*, both austral migrants (Jahn et al., 2002). Thirty kilometers is not an unreasonable distance for a migrant bird to carry seeds, and given the existence of suitable chili habitat between these populations, there appears to be ample opportunity for gene flow between populations, even for a predominantly self-pollinated species such as *C. chacoense* (McLeod et al., 1982).

If changes in capsaicinoid production are instead due to changes in selective pressures acting on fruit phenotypes, it presents a rare opportunity to identify and characterize the selective pressures that favor the production of a major secondary metabolite. We know that birds readily eat both pungent and nonpungent chilies (Tewksbury and Nabhan, 2001; Tewksbury unpublished data). Many aspects of the biotic and abiotic environment likely vary across this 1000-m altitudinal gradient, any one of which might shift in favor of pungent plants at higher elevations. For example, nonpungent fruits in polymorphic populations are much more likely than pungent fruits to be damaged by fungi

(Tewksbury and Levey, unpublished data), and the risk of damage may vary across elevation. In addition, in one polymorphic population (c3), capsaicinoids appear to protect seeds from seed predators when fruits fall directly to the ground (Tewksbury and Levey, unpublished data). All of these results suggest complex interactions among different aspects of fruit phenotype. Fruit removal rates, invertebrate seed predation, and fungal pathogen load may all change with elevation and interact with plant density, which varies by more than an order of magnitude from diffuse low-elevation populations to dense high-elevation populations. Thus, there are potentially many biotic and abiotic selective pressures that could influence the chemical phenotype of chili fruits across this gradient.

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