Title

Multiple lines of evidence for the origin of domesticated chili pepper, Capsicum annuum, in Mexico

Journal

Proceedings of the National Academy of Sciences of the United States of America, 111(17)

ISSN

0027-8424

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Publication Date

2014-04-29

DOI

10.1073/pnas.1308933111

Peer reviewed

Genet Resour Crop Evol (2013) 60:225–232
DOI 10.1007/s10722-012-9827-5

RESEARCH ARTICLE

A new collection of wild populations of *Capsicum* in Mexico and the southern United States

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Received: 25 July 2011 / Accepted: 5 March 2012 / Published online: 20 April 2012

Abstract An exploration and collection mission for wild populations of *Capsicum* was carried out in the fall of 2006 and 2007, in 13 Mexican states and in the U.S. states of Arizona and Texas. The aim of this collection was to expand the number of accessions of wild chile pepper (*Capsicum annuum* var. *glabriusculum* and *Capsicum frutescens*) that are publicly available for research in plant improvement and for subsequent use in an inquiry into the domestication of *C. annuum*. While Mexico and the United States National Plant Germplasm System both have germplasm repositories INIFAP—Instituto Nacional de Investigaciones Forestales—Agrícolas y Pecuarias and USDA GRIN—United States Department of Agriculture's Genetic Resources Information Network) with accessions of *C. annuum* var. *glabriusculum*, the very limited number available, their age, and/or validity of the information attached to many accessions do not allow for extensive research. Four hundred and sixty-six plants were sampled over two field seasons, of which copies of the collection reside in both UAA and at UC Davis. Given the current environment with the intellectual property of varieties of crop plants and, particularly, the extreme restrictions affecting explorations and the official procuring and sharing of germplasm across national borders, this U.S.—Mexico collaboration is one of the few examples of joint U.S.—Mexico germplasm collection efforts.

Keywords *Capsicum* · Crop domestication · Germplasm collection · Mexico · United States · Wild crop relatives

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Introduction

At the inception of crop domestication research, it was recognized that rich plant collections were key to understanding the diversity and origin of crops (Darwin 1883; de Candolle 1884; Vavilov 1926). Plant collection has a long and illustrious history—it is the most basic research method shared by both early botanists and present day plant systematists and is one of the few methodologies that has not changed through time. The fundamental component of crop domestication research, the identification of the putative center of origin, necessitates a thorough collection of representative samples of germplasm from across the geographic range of the crop species and its wild

progenitor (Vavilov 1992). The identification of the putative center of domestication occurs through the analysis of shared polymorphisms between a crop and its wild progenitor, once done through the observation of morphological characteristics, currently uses DNA polymorphisms. However, neither phenotypic nor genetic comparisons cannot be made without representative samples of both the domesticate and its putative wild relative(s). With a rich collection, the geographic location where the shared polymorphisms are located can then be identified as the presumed center of domestication of the crop (e.g., common bean, maize, and pearl millet: (Kwak and Gepts 2009; Matsuoka et al. 2002; Oumar et al. 2008).

These recent advances in crop domestication research have relied on having the appropriate samples—both wild and domesticate. In these works, wild populations from their entire range are screened, searching for the population that is most closely related to the domesticate. In many cases, these collections are built up over time, with years of visits and seed multiplication, and for some of the most economically important species, and deposited in germplasm banks such as at the Centro Internacional de Agricultura Tropical (CIAT) and the Centro International de Mejoramiento de Maiz y Trigo (CIMMYT).

Currently, efforts are underway to resolve the uncertainty associated with the domestication of *C. annuum*, its center of origin, and its relationship to *C. annuum* var. *glabriusculum*, the putative wild ancestor (Pickersgill 1971). Recent advances in molecular identification of the species through DNA techniques—either via SNPs (Jeong et al. 2010) or through a suite of diagnostic microsatellite markers for the genus (Lee et al. 2004; Yi et al. 2006; Minamiyama et al. 2006) are creating tools that were previously unavailable during previous *Capsicum* domestication studies (Loaiza-Figueroa et al. 1989). In fact, some researchers have already begun to put these tools to use (Aguilar-Meléndez et al. 2009). There are publications that detail a number of populations of *C. annuum* var. *glabriusculum* (Hernandez-Verdugo et al. 2001a; Loaiza-Figueroa et al. 1989; Oyama et al. 2006; Perramond 2005; Tewksbury et al. 1999), yet seeds from these populations are not available to other researchers. For many projects, germplasm banks or repositories are the sole sources of samples. The USDA has a very thorough and complete selection of domesticated accessions of *Capsicum*. However, search of the USDA Germplasm Resources Information Network (GRIN) on July 29th, 2009 (USDA 2009) showed only 26 wild accessions of *C. annuum* var. *glabriusculum*. Of these, the following countries of origin were represented by few samples: Costa Rica: one accession; Guatemala: nine accessions; Mexico: 12 accessions; Nicaragua: three accessions; U.S. Outlying Islands: one accession; United States: one accession. On closer inspection, many of these accessions lack geographical coordinates (only three in Mexico are mapped and one of these comes from a market), with only a rough approximation as to their provenance. Additionally, during a preliminary grow-out, the taxonomic status of many of these accessions were put in doubt as they displayed morphological characteristics beyond the phenotypical range of *C. annuum* var. *glabriusculum* (Kraft and Luna Ruiz, personal observation) and many specimens have been re-classified many times by USDA staff botanists or collaborators who see pictures of the flower or fruits on-line via the USDA GRIN database.

The situation with the *Capsicum* national germplasm collection at INIFAP is unclear. Although KHK and JLR had personal communications with Salvador Montes-Hernández, curator of horticultural crops at INIFAP and multiple visits to the offices of the germplasm bank, efforts to obtain *Capsicum* germplasm from the INIFAP collection and efforts to look at the database of samples were not successful to date.

Given the limited number of wild samples that are currently available for research on the domestication and centers of origin of *Capsicum*, it was imperative that such a collection was begun.

The objective of this paper is to detail a two-year collection effort of wild *Capsicum* in Mexico and the southwestern United States. It is hoped that this collection can be the foundation of further research efforts into the ecology of wild *Capsicum* and into the relationship between wild populations and the domesticated chile peppers.

Materials and methods

Assembling the collection

Sampling locations were identified through a mix of methods. Previous studies that identified populations of wild pepper were cataloged (Hernandez-Verdugo et al.

2001a, 2001b; Loaiza-Figueroa et al. 1989; Tewksbury et al. 1999; Votava et al. 2002; Perramond 2005) and cross-tabulated with information obtained from local informants and Mexican collaborators (J. Luna-Ruíz, S. Hernández-Verdugo, T. Estrada, H. Villalón). In addition, the potential species distribution was modeled using FLORAMAP (Jones 1996) using the coordinates identified in these studies. FLORAMAP is a plant habitat modeling program that uses presence data for a species and correlates it to three bioclimatic variables—precipitation, day length and mean temperature. The relative contributions of each bioclimatic variable can be modified, relative to one another, until the most number of presence points have been included in the model. FLORAMAP has a rather large pixel resolution of 18 km × 18 km, or 324 km^2, and therefore was used to identify general regions to target, rather than to identify specific collection locations, which were identified through the use of local informants and previous studies. Areas with high probability of occurrence and that are important for wild chile peppers were identified (areas with red and yellow on) and placed on the itinerary for sampling.

Collecting trips and documentation

In 2006, the itinerary included Arizona, northern and southern Sonora, Sinaloa, Jalisco, Querétaro and Veracruz. In 2007, areas included southern Texas, Nuevo León, Tamaulipas, Yucatán, Chiapas, Oaxaca, and Baja California Sur. Collection trips were timed to coincide with fruiting periods of the plants. Collection trips started in October and terminated in early December. Using a personal vehicle, KHK (and many times with JLR) would drive to the areas identified and search for local informants who could aid in the localization of plants in the area. Species ID were made GPS coordinates of each plant were recorded along with altitude (in meters above sea level) using a Garmin *e*Trex and the geo-political location of the presumed wild specimens (town, municipality, state), and all the ripe fruits collected from a single plant were given a unique ID. Ripe fruits were placed directly into manila envelopes, where exposure to the arid environments would ensure dried fruits in 3–5 days.

Capsicum populations were sampled in chaparral, secondary forests, in human disturbed habitats, and in agro-ecosystems such as coffee and banana plantations. Plants that were found growing in minimally disturbed habitat were considered to be "wild," whereas plants collected from areas that were disturbed and managed by humans, yet not intentionally planted, were considered ruderal or "semi-wild."

On two occasions, collaborators (Sergio Hernandez-Verdugo at the Universidad Autónoma de Sinaloa and Horacio Villalón-Mendoza at the Universidad Autónoma de Nuevo León) shared seed and collection information. A number of Mexican landraces were collected adventitiously as well: chile cora from Nayarit, and guajillo and pasilla from Aguascalientes, which would serve as the domesticated comparisons, as these were purely Mexican varieties, with little production outside of Mexico (Andrews 1995).

A permit to collect and transport seeds of *C. annuum* var. *glabriusculum* was obtained from the state office of SAGARPA in Aguascalientes. Additional permission and clearance was sought from the USDA and U.S. Customs and Immigration. Given the very sensitive atmosphere regarding the use and ownership of germplasm, the collection was divided into two halves. Half currently resides with JLR at the Universidad Autónoma de Aguascalientes, and the other was brought to UC Davis, where it currently resides in the laboratory of PG. Efforts are currently underway to multiply the seed in both locations to make them available to other research teams.

Results

A total of 466 plants were sampled from hillsides, fields, creek beds and home gardens and markets (Fig. 1). The wild *Capsicum* collection was found in a number of habitats and locales—from N32.24° to N16.54°, W116.16° to W88.23° and from 13 to 2,023 masl (see Table 1). We will review in closer detail each of the regions where collections were obtained.

Arizona and Sonora, Mexico

In Arizona and northwestern Mexico, *C. annuum* var. *glabriusculum* is known as *chiltepin* and is gathered and commercialized heavily (Nabhan 1985; Perramond 2005), often fetching prices as high as $70 US dollars per kilo in larger markets such as Hermosillo or Tijuana. Additionally, this region hosts the only protected area specifically designated for the protection of wild crop relatives—the wild chile botanical area (Tewksbury et al.

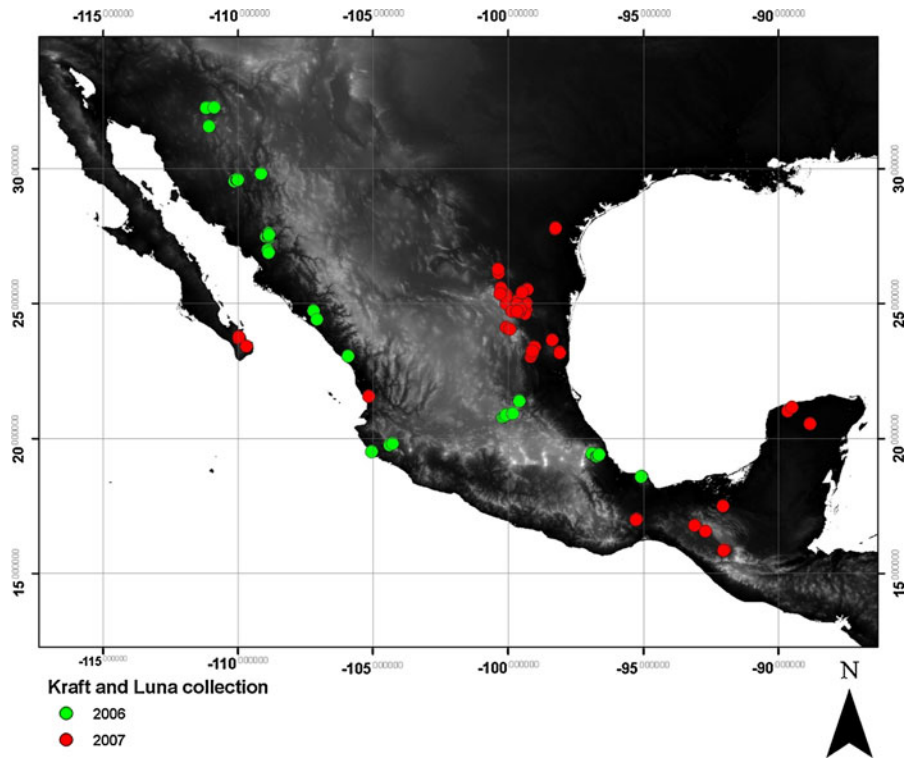

Fig. 1 Map of 2006 and 2007 collections by Kraft and Luna. Points include physical collections and/or collections that were shared from Mexican collaborators

1999). Accessions were collected from the botanical area, in the foothills of the Tumacacori Mountains of southern Arizona. Collections were made in northern Sonora, along the Rio Sonora valley, arguably the capital of wild *Capsicum* commercialization (Perramond 2005), and further south, in the municipality of Alamos in Southern Sonora. These accessions from southern Arizona and Sonora are characterized by small, spherical, erect fruits, with elliptic to ovate slightly pubescent leaves and were found in the understory of larger trees, such as mesquites (e.g., velvet mesquite *Prosopis velutina*, see Fig. 2) and desert hackberry (*granjeno* in Spanish *Celtis ehrenbergiana*) or under columnar cacti (tribe *Pachycereeae*, many species).

Baja California, Sinaloa and Jalisco

Sergio Hernandez-Verdugo of the Universidad Autónoma de Sinaloa provided samples of populations in Sinaloa that have been used in his research on genetic diversity (Hernandez-Verdugo et al. 2001a, 2001b). Sinaloa is known as "Mexico's breadbasket" and is home to the greatest concentration of commercial agricultural operations in the country. The original populations collected by Dr. Hernández-Verdugo of *C. annuum* var. *glabriusculum* have since been plowed up for commercial production of export crops such as tomatoes and cucumbers or for illicit crops such as marijuana (S. Hernandez-Verdugo, personal communication). No other extant populations were found.

In the state of Jalisco, accessions were collected near the town of Autlán de Navarro. Of extreme interest were non-pungent wild accessions. These plants had similar to characteristics as other wild accessions, yet with larger, more oblong fruits and the surprising lack of pungency. It has been postulated that pungency aids in the defense of the plant against seed predation—either by mammals (Tewksbury and Nabhan 2001) or through fungus (Tewksbury et al. 2008). Additional accessions were collected in the Chamela—Cuixmala Biosphere Reserve, a tropical dry forest, managed by the Universidad Nacional Autónoma de Mexico (UNAM). Accessions were found near disturbed areas—forest edges, along the human-made trails.

Accessions were collected in Baja California Sur with the aid of Dra. Lilia Alcaraz Meléndez, from the Centro de Investigaciones Biológicas del Noroeste, S.C. in La Paz. She aided in helping to identify a number of populations that were dispersed across the extreme southern end the peninsula and found in creek

Table 1 Summary of accessions, species found, elevation range and other remarks

Location	Number of accessions	Species found	Elevation range (m)	Other remarks
Arizona (USA), Sonora (MEX)	105	*C. annuum* var. *glabriusculum* (Dunal) Heiser et Pickersgill	237–1,168	Wild harvested, commercialized as *chiltepin*
Baja California and Sinaloa and Jalisco (MEX)	66	*C. annuum* var. *glabriusculum* (Dunal) Heiser et Pickersgill	50–958	Non pungent population found in Jalisco, Sinaloa populations contributed by Dr. Hernández-Verdugo
Querétaro (MEX)	47	*C. annuum* var. *glabriusculum* (Dunal) Heiser et Pickersgill	592–2,023	High altitude populations
Texas (USA), Tamaulipas, Nuevo Leon	109	*C. annuum* var. *glabriusculum* (Dunal) Heiser et Pickersgill	109–495	Commercialized in immature form, *chile pequin*, many donated by Dr. Horacio Villalón
Veracruz	38	*C. frutescens* L.	68–1,070	Various fruit morphologies, locals identified them as invasives
Yucatán, Chiapas, Oaxaca	20	*C. annuum* var. *glabriusculum* (Dunal) Heiser et Pickersgill	18–569	Known as *maax'ic*, in Mayan
Market specimens	81	*C. annuum* L.	N/A	Collected adventitiously and to serve as domestic comparison

beds and low-lying areas. Generally speaking, these accessions collected were morphologically similar (with similar cultural use, but not an extreme commercialization) to those found in Sonora and Arizona.

Querétaro

The only accessions collected in the interior of the country were in the foothills of the Sierra Gorda in Querétaro. Other accessions in the state were obtained at the northeastern extremes of the political boundaries, entering into the fringes of the Huasteca Basin. It was a surprise to both KHK and JLR to have found multiple populations (more than 10 km apart) of wild *C. annuum* var. *glabriusculum* at above 2,000 m a. s. l. All previous published records of these populations occur in elevations less than 1,200 masl. JLR believes these populations are the highest elevation recorded for *C. annuum* var. *glabriusculum*. Fruit that was oblong, with a pronounced point, characterized these accessions collected in Querétaro. The local population living nearby did not commercialize the fruit. These populations were collected under a variety of nurse trees, including some large *Opuntia* spp. cacti (see Fig. 3).

Northeastern Mexico and Texas

In the northeast of Mexico, accessions were collected in Tamaulipas, Nuevo Leon and in U.S. state of Texas. Additionally, Horacio Villalón of the Universidad Autónoma de Nuevo León at Linares, NL, generously shared over 70 accessions. Collections here were made in both human disturbed landscapes—such as pastures and fence lines, in addition to natural landscapes. In this region, the wild pepper is primarily commercialized in its green, unripened form and known by a vernacular name, *chile piquín.* These chiles have a similar ecology to those found in Sonora, often found under nurse trees. However, the plants are much more robust with denser foliage with a reduced internodal space.

Veracruz

Down the eastern coast of Mexico, accessions were collected along the Rio Jacomulco valley, which flows from the flanks of the Sierra Madre Oriental, into the Gulf of Mexico in the state of Veracruz. Accessions

Fig. 2 One of the authors (KHK) with a *chiltepin* under a mesquite tree in Sonora, Mexico

were also collected from the Universidad Nacional Autónoma México's Las Tuxtlas Biological station, located in the tropical wet forest adjacent the Gulf of Mexico. Herbaria records obtained at UNAM had shown the presence of *C. annuum* var. *glabriusculum* in the area, yet many of the individuals found and sampled had morphological characteristics of *C. frutescens* (e.g. very pointed and oblong fruits, multiple fruits per axil, extremely pungent, see Fig. 4), and were often feral—found in disturbed habitats on the peripheries of human settlements. Residents had anecdotes of how this new type of chile, which was much more pungent, had displaced the "chile conguito"—which was described as a *C. annuum* var. *glabriusculum* type. These chiles were not commercialized in any way.

The Yucatán Peninsula Area and Southern Mexico

In the states of Yucatán, Oaxaca and Chiapas, the accessions collected conformed more to the expected morphology of *C. annuum* var. *glabriusculum*, albeit with more oblong fruit. These accessions continued to be found on disturbed habitat, such as fence rows, and roadsides. These areas contain the greatest cultural diversity in Mexico. The lack of truly wild accessions in these areas may reflect that the chiles dispersed adventitiously where humans had already settled; or in contrast, it may reflect that there is a lack of land that are minimally disturbed by man.

In the Yucatan peninsula, the accessions are commercialized in the region and have the local name of *maax'ic*, which is Mayan for "small chile". It is interesting to note the Mayan name for this chile, as there are only a few other varieties in the area with Mayan monikers.

In Chiapas, and Oaxaca, the few accessions that were collected were feral and found on roadsides. There was no sign of commercialization.

Discussion

While the majority of the collection was made in coastal areas, both on the Pacific and Atlantic sides,

Fig. 3 Wild *Capsicum* growing under an *Opuntia* cactus in Querétaro

the major surprise of the collection was the Querétaro populations that were found at over 2,000 m above sea level. These populations were growing just to the south of the foothills of the Sierra Gorda Biosphere reserve. While the fruits were more oblong, they had similar ecological niches to those from Northwestern Mexico—namely they were found growing under nurse trees in a chaparral environment.

The accessions sampled in these two collection trips constitute the most extensive collection of Mexican wild *Capsicum* in existence. This collection is especially deep in the coverage of *C. annuum* var. *glabriusculum* from northwestern Mexico. However, there remain a few areas of interest that should be visited in future collections, namely areas in Northern Mexico that were not collected—Chihuahua, the Huasteca basin to name a few. The gradient between wild, semi-wild, and domesticated is quite fine and collectors in the field are challenged to identify the different species of *Capsicum*. Given the uncertainty with the taxonomic origins of feral peppers from Veracruz, and the suggestion that the domestication of *C. annuum* occurred in northeastern Mexico (Loaiza-Figueroa et al. 1989), the Gulf Coast of Mexico should be a target for future collection and investigation. If well maintained and renewed, this collection will allow for many future studies on the genetic differentiation of these populations for future plant breeding efforts in *Capsicum*, as well as ethnobotanical studies. We are currently working towards making small seed samples available after its multiplication in both Aguascalientes, Mexico and Davis, California.

Fig. 4 Individuals of *C. frutescens* in Veracruz, Mexico, with distinct fruit morphologies

Acknowledgments KHK, JLR and PG were funded by two different UCMEXUS grants. KHK was funded by a García–Robles Fulbright fellowship. Thanks to Mexican collaborators: Dr. Sergio Hernández-Verdugo, Dr. Horacio Villálon, Dr. Lilia Alcaraz, Dr. Francisco Cora. KHK would like to thank Heather Zornetzer for her invaluable assistance during field collection.

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