Unraveling the Species Dilemma in *Capsicum frutescens* and *C. chinense* (Solanaceae): A Multiple Evidence Approach Using Morphology, Molecular Analysis, and Sexual Compatibility

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ABSTRACT. The species status of two morphologically closely related species, *Capsicum frutescens* L. and *C. chinense* Jacq., was investigated using typological, phylogenetic, and biological species concepts. Diagnostic morphological differences, randomly amplified polymorphic DNA (RAPD) marker-based cluster analysis, and hybrid analyses were used to delimit *C. frutescens* and *C. chinense*. In many cases two morphological characters, calyx constriction and flower position, can separate accessions of *C. frutescens* from *C. chinense*. The RAPD-based analysis clearly separated accessions of *C. frutescens* and *C. chinense* into two distinct groups. The average genetic similarity within *C. frutescens* and *C. chinense* accessions was 0.85 and 0.8, respectively, whereas the average genetic similarity between *C. frutescens* and *C. chinense* accessions was only 0.38. The progenies obtained from *C. frutescens* and *C. chinense* hybridization had reduced fertility. Based on these evidences, *C. frutescens* and *C. chinense* represent two morphologically diagnosable, phylogenetically distinct, and reproductively isolated species.

Two *Capsicum* L. species, *C. frutescens* and *C. chinense*, are morphologically similar. One of the most lingering questions in *Capsicum* taxonomy is whether *C. frutescens* and *C. chinense* are two different species or botanical varieties of the same species. A species is defined as a group of actually or potentially interbreeding natural populations that are reproductively isolated from other such groups (Mayr, 1970). On the other hand, botanical variety is a distinct morphological subgroup within a species. It has been argued that *C. frutescens* and *C. chinense* are separate species (Smith and Heiser, 1957), and conversely that they are both one species, but different varieties (Eshbaugh et al., 1983). However, the genetic relationship between these two species is still in speculation.

Capsicum frutescens has maintained its species recognition since Linnaeus (1753). He described it as having paired pedicels per node and a frutescent stem, i.e., perennial plant habit. Shaw and Khan (1928) added waxy greenish-white corolla as a unique character of *C. frutescens*. In addition, Standley (1931) stated that *C. frutescens* has small fruits and the pedicel is generally longer than the fruit.

The name *C. sinense* (*C. chinense*) was first given by Jacquin in 1776. But this recognition was unknown until Smith and Heiser (1957) re-examined these materials and suggested separating Jacquin's *Capsicum sinense* from *C. frutescens* and renaming it as *C. chinense*. Smith and Heiser (1957) described *C. chinense* as having glabrous leaves and stem, with ovate to ovate lanceolate leaves. There are two to five flower buds per node, pedicels are declinate and relatively short and thick. In addition, it has a calyx without teeth, but with a marked constriction between the pedicel

and calyx. However, there are accessions that possess one or a few diagnostic morphological characters from one species and the rest of the characters from the other species, creating difficulties in species assignment. Smith and Heiser (1957) compared these two species systematically for the first time, and concluded that *C. frutescens* and *C. chinense* could be distinguished morphologically. Their hybridization experiment between *C. frutescens* and *C. chinense* resulted in a very low seed set as well as a very low pollen viability in plants of the F₁ generation. The F₁ plants produced nonviable seeds. As more accessions of both species were studied, they were found to be closely related. The relationship appeared to be so close that separate species treatment was eventually questioned by many researchers (Eshbaugh, 1993; McLeod et al., 1983; Pickersgill, 1988). The conflicting reports have confounded the biological and taxonomic relationships between these two species.

Capsicum frutescens and *C. chinense* are sympatrically distributed throughout their range in Peru, Brazil, Colombia, and Bolivia (Pickersgill, 1971). A numerical taxonomic analysis using morphological characters could not separate the accessions of *C. frutescens* and *C. chinense* according to species (Pickersgill et al., 1979). Similarly, use of various enzyme loci also could not distinguish *C. annuum*, *C. frutescens*, and *C. chinense* accessions (Jensen et al., 1979). Egawa and Tanaka (1984) reported fertile interspecific hybrids between *C. frutescens* and *C. chinense* with regular meiosis.

Herein, we describe a more comprehensive examination of *C. frutescens* and *C. chinense* using morphological, phylogenetic, and sexual compatibility as evidence. The morphological, phylogenetic, and sexual compatibility approaches correspond with typological, phylogenetic, and biological species concepts, respectively. Morphological characters were examined to determine whether fixed differences exist between *C. frutescens* and *C. chinense* (typological species concept). Randomly ampli-

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fied polymorphic DNA (RAPD) marker-based cluster analysis determined if *C. frutescens* and *C. chinense* are two separate species, based on the phylogenetic species concept. The sexual compatibility between *C. frutescens* and *C. chinense* was investigated to understand whether *C. frutescens* and *C. chinense* are reproductively isolated (biological species concept).

Materials and Methods

PLANT MATERIALS. A total of 191 accessions of *C. frutescens* and 223 accessions of *C. chinense* were acquired from the USDA, Regional Plant Introduction Station, Griffin, Ga., and 20 accessions of *C. frutescens* were obtained from the New Mexico Capsicum Accession (NMCA) collection at New Mexico State Univ. These accessions represent collections from various regions of the World.

MORPHOLOGY. Morphological data were collected from plants in field plots for the following diagnostic morphological characters: leaf texture, number of flowers per axil, flower position, calyx margin, calyx constriction, corolla color, anther color, filament color, fruit persistence, and fruit size. The morphological data from the field observations were analyzed by discriminant function analysis (SAS Institute, 2001) to determine if any combination of characteristics could separate these two species.

RAPD ANALYSIS. RAPD produced polymorphic DNA markers. Accessions were selected for RAPD analysis based on morphological characterizations. Plants fitting the current taxonomic description of *C. frutescens* and *C. chinense* and showing uniformity in the field were selected for RAPD analysis. Among the 75 accessions selected for the analysis, 41 accessions were *C. frutescens* and the other 34 accessions were *C. chinense*. In addition, 'Greenleaf Tabasco', an interspecific hybrid between *C. frutescens* and *C. chinense*, was also included. This accession possesses most of the characters of *C. frutescens*, but contains some features, such as a constricted calyx and virus resistance from *C. chinense* (Greenleaf et al., 1970). One accession of *C. annuum* L. was used as an outgroup. A list of accessions including the PI number and the country of origin is provided in Table 1.

DNA ISOLATION. From each accession, six plants were randomly selected. Collection of tissue samples, DNA isolation, and DNA quantification methods were as described by Votava and Bosland (2002). After quantification, the DNA was diluted to 10 ng·mL⁻¹ in a TE buffer (10 mM Tris and 0.1 mM EDTA) for further use.

PCR FOR RAPD ANALYSIS. The cellular DNA was amplified using the polymerase chain reaction method (PCR) to generate RAPD markers (Williams et al., 1993). PCR was performed on a Geneamp 9600 PCR system (Perkin Elmer Corp., Foster City, Calif.). The decamer primers obtained from Operon Technology (Alameda, Calif.) were diluted in distilled water. Primers used in this analysis were: OPAA-11, OPE-05, OPM-04, OPA-04, OPA-07, OPE-12, OPC-06, OPA-16, OPE-02, OPA-17, OPF-05, OPM-09, and OPA-02. The total volume of each PCR reaction was 20 mL containing: 20 ng template DNA, 10 mM Tris-HCl, 10 mM KCl, 356 mM MgCl₂, 101 mM each dNTP, 2 units AmpliTaq polymerase (Stoeffel fragment), and 0.24 mM primer.

The PCR thermal profile was as described by Gallego and Martínez (1997). There was a preliminary step of 2 min at 94 °C for the template denaturation. The first 10 cycles consisted of 30 s at 94 °C, 1 min at 55 °C, and 4.5 min at 72 °C. The primer annealing temperature (55 °C) was reduced by 1 °C in each cycle.

The next 25 cycles also followed the same thermal profile except the annealing temperature was held constant at 45 °C. There was a hold of 1 min at 72 °C for the final extension. In order to verify the reliability and repeatability of the RAPD, 10% of the total samples were duplicated. If each pair of duplicated samples failed to produce identical bands, the gel was discarded. There was always one blank sample as a negative control, which contained all reagents for the PCR reaction, except the DNA template.

The PCR product was separated by electrophoresis in a 1.85% agarose gel in TBE buffer (89 mM Tris, 89 mM Borate, and 2.5 mM EDTA), stained with ethidium bromide, and viewed under ultraviolet light. The computer software alpha imager gel documentation system (Alpha Inno-tech Corp., St. San Leandro, Calif.) generated a digital image of the gel. The presence or absence of RAPD bands was scored manually in a gel image analysis software, RFLP-Pro (DNA Pro Scan, Nashville, Tenn.). If a RAPD band was present in a particular accession, it was scored as "1"; if the band was not present it was scored as "0." The frequency for each RAPD marker was calculated as a proportion of the presence of a particular RAPD marker, divided by the total number of accessions included in the analysis for that species.

Distribution of RAPD frequency among the accessions of each species was plotted on a graph. The unweighted pair-group with arithmetic averages (UPGMA) method was used in the cluster analysis (Sneath and Sokal, 1973), which was available in the computer software numerical taxonomy and multivariate analysis system, NTSYS (Rohlf, 1998). The similarity coefficient for all possible pairwise combinations was calculated using NTSYS. The Simqual program of NTSYS uses an algorithm to calculate the Dice similarity coefficient for all possible pairwise combinations (Dice, 1945). The value of similarity coefficient lies between "0" and "1." The pairwise similarity coefficient matrix was used for the cluster analysis using UPGMA method. The result of the cluster analysis was represented in the form of a dendrogram.

HYBRIDIZATION STUDY. For the hybridization study, five *C. chinense* and five *C. frutescens* accessions were selected based on the results of the RAPD analysis to maximize the genetic distance among the hybridizing parent. All possible combinations including reciprocal hybridizations were made. The hybridizations were accomplished in a climate-controlled greenhouse. The flowers were emasculated at the white bud stage. A fresh opened flower was detached from the male parent and the dehiscent anthers were rubbed on the stigma surface of the emasculated flower. Initially five hybridizations per combination were attempted, but if the flower aborted after pollination, the hybridization was repeated. Up to 15 hybridizations were made for some combinations. The mature fruits were harvested and the number of seeds set in each fruit was recorded.

ANALYSIS OF F₁ PLANTS. The F₁ seeds obtained from the hybridizations were germinated in the laboratory. The seedlings were transplanted into pots in the greenhouse. Three F₁ plants from each hybrid combination were selected for pollen viability testing. Three flowers prior to anthesis were collected from each plant. Anthers were carefully removed with forceps and placed on a microscope slide. A drop of 0.75% aceto-carmin (Carolina Biological Supply Co., N.C.) was added to each slide. The anthers were ruptured with the forceps to release the pollen grains. The slide was briefly exposed to a flame and spread with forceps to allow better staining. The total number of pollen grains and the total number of viable pollen grains in a microscope field were counted. Three counts were taken per slide.

Table 1. List of *Capsicum frutescens* and *C. chinense* accessions used in the RAPD analysis.

Accession ID	Origin ^y	Species	Accession ID	Origin ^y	Species
PI ^z 939497	Costa Rica	<i>C. frutescens</i>	NMCA 40029	Columbia	<i>C. frutescens</i>
PI 439498	Costa Rica	<i>C. frutescens</i>	NMCA 40028	SA218	<i>C. frutescens</i>
PI 439502	Costa Rica	<i>C. frutescens</i>	Greenleaf Tabasco	United States	<i>C. frutescens</i>
PI 439506	Cuba	<i>C. frutescens</i>	PI 213918	Bolivia	<i>C. chinense</i>
PI 439508	El Salvador	<i>C. frutescens</i>	Grief 9269	Costa Rica	<i>C. chinense</i>
PI 439509	Fiji	<i>C. frutescens</i>	Grief 9304	Colombia	<i>C. chinense</i>
PI 439514	Mexico	<i>C. frutescens</i>	PI 215734	Peru	<i>C. chinense</i>
PI 260479	Peru	<i>C. frutescens</i>	PI 224424	Costa Rica	<i>C. chinense</i>
PI 273427	United States	<i>C. frutescens</i>	Grief 9238	Costa Rica	<i>C. chinense</i>
PI 281347	India	<i>C. frutescens</i>	Grief 9302	Colombia	<i>C. chinense</i>
PI 281396	Mexico	<i>C. frutescens</i>	PI 224446	Costa Rica	<i>C. chinense</i>
PI 355395	Ecuador	<i>C. frutescens</i>	PI 241668	Ecuador	<i>C. chinense</i>
PI 355808	Ecuador	<i>C. frutescens</i>	PI 238052	Peru	<i>C. chinense</i>
PI 358811	Malaysia	<i>C. frutescens</i>	PI 238047	Guyana	<i>C. chinense</i>
PI 358968	Ecuador	<i>C. frutescens</i>	PI 593612	United States	<i>C. chinense</i>
PI 238059	Guatemala	<i>C. frutescens</i>	PI 439455	Peru	<i>C. chinense</i>
PI 238060	Suriname	<i>C. frutescens</i>	PI 439448	Peru	<i>C. chinense</i>
PI 439492	Brazil	<i>C. frutescens</i>	PI 439447	Peru	<i>C. chinense</i>
PI 439490	Brazil	<i>C. frutescens</i>	PI 439442	Peru	<i>C. chinense</i>
PI 439489	Brazil	<i>C. frutescens</i>	PI 439426	Costa Rica	<i>C. chinense</i>
PI 39488	Bolivia	<i>C. frutescens</i>	PI 439476	United States	<i>C. chinense</i>
PI 439494	Costa Rica	<i>C. frutescens</i>	PI 439463	Peru	<i>C. chinense</i>
PI 439493	Colombia	<i>C. frutescens</i>	PI 439462	Peru	<i>C. chinense</i>
PI 439491	Brazil	<i>C. frutescens</i>	PI 439460	Peru	<i>C. chinense</i>
PI 224416	Costa Rica	<i>C. frutescens</i>	PI 438614	Mexico	<i>C. chinense</i>
PI 195296	Guatemala	<i>C. frutescens</i>	PI 438630	Mexico	<i>C. chinense</i>
PI 593613	United States	<i>C. frutescens</i>	PI 438648	Mexico	<i>C. chinense</i>
PI 586675	United States	<i>C. frutescens</i>	PI 438643	Mexico	<i>C. chinense</i>
PI 441686	Brazil	<i>C. frutescens</i>	PI 260477	Peru	<i>C. chinense</i>
PI 441653	Brazil	<i>C. frutescens</i>	PI 260471	Peru	<i>C. chinense</i>
PI 441647	Brazil	<i>C. frutescens</i>	PI 260558	Peru	<i>C. chinense</i>
PI 441645	Brazil	<i>C. frutescens</i>	PI 260522	Peru	<i>C. chinense</i>
PI 441642	Brazil	<i>C. frutescens</i>	PI 260492	Bolivia	<i>C. chinense</i>
Grif 9331	Costa Rica	<i>C. frutescens</i>	PI 315010	Peru	<i>C. chinense</i>
PI 593614	Philippines	<i>C. frutescens</i>	PI 315019	Peru	<i>C. chinense</i>
NMCA 40040	Zimbabwe	<i>C. frutescens</i>	PI 315021	Peru	<i>C. chinense</i>
NMCA 40035	Ecuador	<i>C. frutescens</i>	PI 315026	Peru	<i>C. chinense</i>
NMCA 40030	Columbia	<i>C. frutescens</i>			

^zUSDA, Plant Introduction Number.^yCountry from where the material was originally collected.

Results

MORPHOLOGICAL CHARACTERIZATION. Morphological characterization was completed for a total 301 accessions. *Capsicum frutescens* and *C. chinense* accessions were assigned to the corresponding species, based on morphological characteristics. According to the morphological characteristics, 70% of the accessions agreed with the species assignment at the germplasm repository. Among the accessions assigned to *C. frutescens* in the germplasm repository, 8% belonged to *C. chinense* after field observations. Similarly, among the *C. chinense* accessions, 9% were found to belong to *C. frutescens*. In addition, 8% of the accessions possessed an intermediate phenotype, making it difficult to be assigned to either *C. frutescens* or *C. chinense* species based morphology.

Variations were observed on fruit related characteristics such as fruit shape, size, and color. There were some accessions in *C. frutescens* that had large, persistent (nondeciduous) fruits. Accessions PI 439498, PI 439502, PI 238059, PI 238060, and PI

195296 fell into this category. These accessions were originally collected in Costa Rica and Guatemala. The large, pendent fruit accessions suggest that some accessions of *C. frutescens* exist in a domesticated form in addition to the cultivated 'Tabasco'.

DISCRIMINANT FUNCTION ANALYSIS. Discriminant function analysis evaluated the ability of diagnostic characters to classify

Table 2. Results of discriminant analysis in *Capsicum* using calyx constriction and flower position.

Species	<i>C. chinense</i>	<i>C. frutescens</i>	Total
From			
<i>C. chinense</i>	32 ^z (94.12) ^y	2 (5.88)	34 (100.00)
<i>C. frutescens</i>	2 (5.00)	38 (95.00)	40 (100.00)
Total	34 (45.95)	40 (54.05)	74 (100.00)

^zNumber of observations.^yPercent classified into species.

Fig. 1. Flower position as a diagnostic character to identify *Capsicum frutescens* and *C. chinense*. *Capsicum frutescens* (left) produces erect flowers and *C. chinense* (right) produces pendent flowers.

the accessions to their corresponding species. From this analysis, calyx constriction and flower position were found to be the most useful combination of diagnostic characters to differentiate the two species (Table 2). When these two characteristics were used in the analysis, 95% of the *C. frutescens* accessions could be correctly identified. Similarly, 94% of the *C. chinense* accessions could be correctly identified with the same two diagnostic characters.

The character erect flower position (Fig. 1) was present in 98% of the *C. frutescens* accessions and only in 8% of the *C. chinense* accessions. The second character separating these two species was the presence of a distinct circular constriction between the pedicel and the calyx in *C. chinense*. Calyx constriction was present in more than 92% of the *C. chinense* accessions, whereas in *C. frutescens* it was found in only 5% of the accessions.

Five percent of the *C. chinense* accessions had a single flower per axil, 5% had a mixture of single and double flowers per axil on the same plant, and 90% had two or more flowers per axil. Among the *C. frutescens* accessions, 57% of the plants had a single flower per axil, 33% had a single and two flowers, and 10% had two or more flowers per axil.

DISTRIBUTION OF RAPD MARKERS. The PCR method generated RAPD markers among the accessions of *C. frutescens* and *C. chinense* (Fig. 2). The RAPD markers varied in size from 200 bp to 2000 bp. Using 13 primers, 106 reproducible polymorphic RAPD markers were obtained among the 75 accessions.

The majority of RAPD markers were found to be present either in a very few accessions or in the majority of the accessions in both species (bimodal distribution). If a RAPD marker frequency was greater than 0.95 in one species and <0.05 in the other species, that marker was considered as fixed (Wiens and

Servedio, 2000). Two RAPD markers were found to be fixed in *C. frutescens* (OPA7₇₅₀ and OPM9₁₉₅₀) and three RAPD markers were found to be fixed in *C. chinense* (OPAA11₁₂₂₅, OPAA11₇₀₀, and OPC6₃₂₀). Furthermore, *C. chinense* had 25 unique RAPD markers that were not present in *C. frutescens*. However, 17 of these unique RAPD markers in *C. chinense* had a frequency of <0.05. *Capsicum frutescens* had seven unique RAPD markers, but five of them had a frequency of <0.05.

GENETIC SIMILARITY AND CLUSTER ANALYSIS.

The genetic similarities within a species and between the species were calculated by averaging the Dice's similarity coefficient for pairwise comparison. The average genetic similarity between *C. frutescens* and *C. chinense* was 0.38, whereas the average genetic similarity within *C. frutescens* was 0.85 and within *C. chinense* was 0.86.

The cluster analysis results were graphically represented in the form of a dendrogram (Fig. 3). There were two clusters, one containing all the *C. frutescens* accessions, and the other containing all the *C. chinense* accessions. The RAPD-based cluster analysis placed 'Greenleaf Tabasco' in the *C. frutescens* group indicating high genetic similarity (Fig. 3). But, it was distinctly separated from rest of the *C. frutescens* accessions occupying an intermediate position between *C. frutescens* and *C. chinense* group.

SEXUAL COMPATIBILITY. In the sexual compatibility study, five accessions of *C. frutescens* (PI 439497, PI 439498, PI 939489, PI 441642, and PI 195296) and five accessions of *C. chinense* (PI 213918, Grif 9269, Grif 9302, PI 438648, and PI 315026) were tested. A total of 819 hybridizations were performed, 352 of which were between species, and 467 of which were within species. From 93 hybridization combinations, 87 combinations developed fruits with seeds, and six combinations developed fruits without seeds. The paired *t* test of reciprocal hybridization was not significant ($P = 0.80$), indicating the absence of unidirectional incompatibility between these two species. Selfing in *C. frutescens* gave only a 15% fruit set, whereas the hybridization among the accessions of *C. frutescens* produced a 36% fruit set. The difference between selfing and sibbing the plants indicates the presence of a weak self-incompatibility system in *C. frutescens*.

COMPARISON OF FERTILITY IN F₁. The pollen viability of F₁ plants derived from the interspecific hybridizations was very low as compared to the intraspecific hybrid plants. The pollen grains

Fig. 2. Gel image showing RAPD fragments amplified by a decamer primer in *Capsicum frutescens* and *C. chinense* accessions. Lanes 1, 34, and 51 size marker. The arrowhead represents the fragment size; lanes 2–6, 24–33, and 35–39 *C. frutescens*; lanes 9–23, 40–47, and 49–50 *C. chinense*; lanes 48 *C. annuum*; lanes 7–8 control.

Fig. 3. Dendrogram showing genetic relationship among the accessions of *Capsicum frutescens* and *C. chinense* as revealed by RAPD markers. **A** = *C. frutescens*; **B** = *C. chinense*; Δ = Greenleaf Tabasco; ★ = included in hybridization.

of *C. frutescens* and *C. chinense* were similar in shape, size, and appearance under the microscope. The aceto-carmin staining technique successfully discriminated normal, viable pollen from nonviable pollen. The viable pollen appeared as large, smooth, bright red, turgid, and triangular structures (Fig. 4). Dead pollen grains appeared very small with a wrinkled surface, probably from the result of cell collapse. Pollen viability was 28% in interspecific hybrid plants, and 75% in intraspecific hybrids.

Discussion

In most cases, *Capsicum frutescens* and *C. chinense* could be identified by the combination of two diagnostic morphological characters: calyx constriction and flower position. The presence of species-specific RAPD markers also provided evidence that fixed differences exist between the two species at the molecular level. *Capsicum frutescens* and *C. chinense* represent two diag-

Fig. 4. Microscope image of viable and nonviable pollen grains from *Capsicum frutescens* × *C. chinense* plants. 4-a. Viable (top) and non-viable (bottom) pollen grains at high magnification (100×). 4-b. Nonviable pollen grains (top) and viable pollen grains at low magnification (20×).

nosable clusters based on shared genetic markers. The result of the cluster analysis represented in the form of a dendrogram has clearly separated each species into separate clusters. The RAPD data did not detect any intergradations between the members of *C. frutescens* and *C. chinense* even though this phenomenon has been previously reported (Jensen et al., 1979; McLeod et al., 1983; Pickersgill, 1988). An explanation for such intergradation could be the selection of plant materials. If interspecific hybrids had been included in this study, then intergradation may have been seen. In addition, some *C. chinense* accessions in the past have been mislabeled as *C. frutescens* because the species name, *C. chinense*, was not being used.

The genetic similarity within *C. frutescens* and *C. chinense* accessions was greater than the genetic similarity between the *C. frutescens* and *C. chinense* accessions. The average genetic similarity coefficient for the pairwise comparisons between the accessions of *C. frutescens* and *C. chinense* was 0.38. Similar results have been reported in other plant species. In *Amaranthus*, the average interspecific genetic similarity was 0.36 as estimated by RAPD markers (Chan and Sun, 1997). The average genetic similarity between the wild and cultivated species of *Trifolium* L. was 0.35, when calculated from isozyme markers (Lange and SchifinoWittmann, 2000). When intraspecific genetic variation was compared, *Capsicum frutescens* and *C. chinense* possessed similar levels of genetic variation. The average genetic similarity coefficient among the accessions of *C. frutescens* was 0.85 and 0.86 among *C. chinense*. Accessions of *C. annuum* var. *annuum* from the center of diversity, i.e., Mexico, have a lower average genetic similarity of 0.75 (Baral and Bosland, 2002).

The RAPD analysis did not support the hypothesis that *C. frutescens* is the primitive form and ancestor of *C. chinense* (Eshbaugh et al., 1983). If *C. chinense* was derived from *C. frutescens*, *C. chinense* accessions would form a cluster nested within *C. frutescens*. However, the data clearly showed parallel genetic diversity in *C. chinense* accessions, which was independent of *C. frutescens*.

Capsicum chinense and *C. frutescens* accessions with intermediate phenotypes can be explained by introgressive hybridization. With introgressive hybridization, genes of one species move

into another through the process of interspecific hybridization followed by successive backcrosses to one of the parents (Anderson, 1949). Natural hybridizations between two species have been reported in many plant species such as in *Helianthus* L. (Rieseberg, 2000), *Iris* L. (Arnold and Bennett, 1993), *Pinus* L. (Wang et al., 1990), and *Stephanomeria* Nutt. (Gallez and Gottlieb, 1982). The cultivar Greenleaf Tabasco offered an excellent example of introgression. This particular cultivar was developed by interspecific hybridization between *C. frutescens* and *C. chinense* followed by repeated backcrossing to *C. frutescens* (Greenleaf et al., 1970). 'Greenleaf Tabasco' resembles *C. frutescens*, but also possesses a few morphological characters of *C. chinense*. The RAPD analysis revealed that 'Greenleaf Tabasco' indeed possessed some DNA from *C. chinense*, as indicated by the presence of *C. chinense* specific RAPD markers. Thus, the *Capsicum* accessions with intermediate phenotypes represent introgressive hybridization, not intraspecific variation.

Capsicum frutescens and *C. chinense* are reproductively isolated. The development of partial sterility in the interspecific hybrid progenies of *C. frutescens* and *C. chinense* provided evidence for reproductive isolation. The pollen viability of F_1 progenies derived from the interspecific hybridizations was 28%, whereas the pollen viability of the F_1 progenies derived from the intraspecific hybridization was 75%. In addition, the interspecific hybrid plants generally had lower fruit set. Embryo abortions were clearly visible as small brown scars on the placental surface of the fruits. Some plants even failed to produce fruit with seeds. The reduced pollen viability of interspecific hybrids has been reported in other crops. The pollen viability of the interspecific hybrid between *Nuphar microphylla* Fernald and *N. variegata* Engelm. ex Clinton was 23% (Padgett et al., 1998). The pollen viability of interspecific hybrid among *Helianthus* species ranged from 11% to 42%. Thus, there appears to be a genetically controlled mechanism(s) that is preventing gene flow between *C. frutescens* and *C. chinense*.

Morphological observations, RAPD-based cluster analysis, and sexual compatibility provided evidence that *C. frutescens* and *C. chinense* are distinct species. Whether the species concept used is typological, phylogenetic, or biological, they all unequivocally support the preposition that these two groups of plants are separate species. Furthermore, the RAPDs have explanatory power and can be used as a basis for establishing species identification. Two particularly important features of RAPDs were ability to do the analysis before the plant flowers, and the ability to detect interspecific introgression. The method clearly demarcates those accessions having risen from interspecific introgression, and may be useful in conservation genetics and crop evolution.

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