Genetic Structure and Phylogenetic Relationships of *Capsicum chinense*

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ABSTRACT. *Capsicum chinense* is commercially the most important pepper species grown in the Caribbean. It is popularly used to impart pungency and flavor to Caribbean cuisine. However, unlike *Capsicum annuum*, which is the most commercially exploited domesticated species internationally, *C. chinense* has not been methodically collected or characterized for systematic improvement through plant breeding. The objectives of the study were to assess the diversity of *C. chinense* and its structure within the Caribbean basin and to determine its phylogenetic relationship to groups within South America. DNA isolated from 201 accessions of *C. chinense*, representing geographical regions where the species is found, were amplified using arbitrary primers to generate 138 polymorphic and reproducible random amplification of polymorphic DNA (RAPD) markers. Nei's and Shannon's diversity indices for *C. chinense* (0.28 and 0.419, respectively) were higher in South America compared with Central America or the Caribbean, corresponding to its putative center of diversity. The study showed the existence of three phylogenetic clusters within *C. chinense*. The largest cluster consisted of accessions from the Upper Amazon region, the Guianas including Venezuela, and the Lesser Antilles of the Caribbean. The other major cluster was represented by accessions principally from the Lower Amazon region. Another distinct but small cluster consisted of samples solely from the Greater Antilles of the Caribbean. The discovery of the three phylogenetic clusters within *C. chinense* may have potential for exploiting heterosis in breeding. The implications of the findings to the understanding of the phylogenetic origin and distribution of *C. chinense* are discussed.

Capsicum peppers are popularly grown around the world as a pungent condiment. The genus name *Capsicum* is believed to have been derived from the Greek word capsicon, which in turn was derived from the Latin root kapetin, which means “to bite” in reference to the fruit's pungency (Weiss, 2002). *Capsicum* (Solanaceae family) consists of 33 accepted wild species and five domesticated species (U.S. Department of Agriculture, 2012): *C. annuum* (chile and sweet pepper types), *C. chinense* (habanero types), *C. frutescens* (tabasco types), *C. pubescens* (rocoto pepper), and *C. baccatum* (aji or peruvian pepper). *Capsicum* species originated in the Neotropics, which extends from southern Mexico through Central America and northern South America to southern Brazil (Pickersgill, 1971), although it is popularly grown and consumed in many parts of the world. *C. chinense* is of Amazonian origin and is indigenous to South America and the Caribbean (Eshbaugh, 1993; Heiser, 1976).

Although *C. annuum* is economically the most important worldwide, market surveys indicate that West Indian *C. chinense* cultivars such as Scotch Bonnet and West Indies Red sell for a premium price in European and North American markets (Cooper et al., 1993). The popularity of the latter has been attributed to its high pungency and distinct flavor profile. Furthermore, the migration of Caribbean people to metropolitan countries around the world, taking with them their unique cultures and cuisines, has created niche markets for *C. chinense*, commonly referred to as ethnic markets.

Despite its importance, productivity of *C. chinense* [10 to 20 Mg·ha⁻¹ (Stewart, 2003)] is low in the Caribbean compared with reported yields for *C. annuum* [30 to 60 Mg·ha⁻¹ (Garcia

et al., 2002)]. Chief among the reasons for low yield are the use of uncharacterized landraces by farmers in the Caribbean. In contrast, *C. annuum* has undergone extensive breeding throughout the world with numerous high-yielding cultivars including hybrids available in the market (Geleta and Labuschagne, 2004; Geleta et al., 2004; Hari et al., 2005; Hundal and Dhall, 2005; Khalil et al., 2002; Patel et al., 2004). Heterosis breeding has been popularly used to improve yields in *C. annuum*, where fruit yield, earliness, and plant height have all shown significant heterosis. However, heterosis breeding requires a thorough understanding of the structure of diversity and the level of heterosis that can be derived for agronomically important traits by intercrossing distinct phylogenetic groups.

During the past decade, molecular markers have been used to distinguish among *Capsicum* species or cultigroups (Baral and Bosland, 2004; Toquica et al., 2003), resolve taxonomic uncertainties (Rodriguez et al., 1999; Toquica et al., 2003), and to assess the genetic diversity in selected populations or germplasm collections (Baral and Bosland, 2002; Buso et al., 2003; Hernandez-Verdugo et al., 2001; Lanteri et al., 2003; Rodriguez et al., 1999; Votava et al., 2005).

Early studies investigating genetic diversity in the genus (wild and domesticated species) used protein- or allozyme-based molecular markers (Loaiza-Figueroa et al., 1989; Panda et al., 1986). These were subsequently replaced by DNA-based molecular markers such as restriction fragment length polymorphism [RFLP (Lefebvre et al., 1993; Prince et al., 1995)], RAPD (Baral and Bosland, 2002, 2004; Buso et al., 2003; Kang et al., 1997; Prince et al., 1995; Rodriguez et al., 1999; Votava and Bosland, 2001; Votava et al., 2005), and amplified fragment length polymorphism [AFLP (Guzmán et al., 2005; Lanteri et al., 2003; Paran et al., 1998; Toquica et al., 2003)].

Primers to amplify 46 microsatellite loci were reported (Lee et al., 2004) using an interspecific cross between *C. annuum*

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'TF68' (Seminis Korea, Seoul, South Korea) and *C. chinense* (cultivar Habanero). Additional microsatellites have been described in interspecific linkage maps (Yi et al., 2006). Microsatellites have been found to be highly variable and codominant; therefore, their information value is high compared with other markers such as RFLPs, AFLPs, and RAPDs. Microsatellites were not used in this study as a result of their unavailability at the time of this work.

The objectives of this study were therefore to use RAPD-based markers to determine the genetic structure within accessions sampled and to estimate the diversity within the groups identified.

Materials and Methods

GERMPLASM COLLECTION AND ESTABLISHMENT. A total of 201 accessions of *C. chinense* from the University of the West Indies germplasm collection were used in this study (Table 1). These accessions were grouped into (or represent germplasm originally obtained from) seven geographical areas: Trinidad and Tobago (79), Greater Antilles (14), Lesser Antilles (19), Central America (11), Upper Amazon (24), Lower Amazon (28), and Guianas including Venezuela (26). For the purposes of this study, the Greater Antilles refers to Caribbean islands such as Jamaica, Cuba, Puerto Rico, and the Bahamas, whereas the Lesser Antilles comprises islands such as Barbados, St. Lucia, and Guadeloupe. In addition, three accessions each of *C. annuum* and *C. baccatum* were included as outgroups in the study for rooting of dendrograms.

MORPHOLOGICAL CHARACTERIZATION. Accessions were characterized for taxonomic characteristics as outlined in the taxonomic key (International Plant Genetic Resources Institute, 1983). These characteristics included for instance the number of flowers per node, flower position, flower color, and the presence of an annular constriction on the peduncle (Table 1). These characters were used to confirm the species identity of the putative *C. chinense* accessions in the collection.

DNA EXTRACTION, POLYMERASE CHAIN REACTION CONDITIONS, AND RANDOM AMPLIFIED POLYMORPHIC DNA BAND SCORING. A sample of 3 g leaf material per accession was pulverized in liquid nitrogen and DNA extracted using a standard protocol (Prince et al., 1997). Extracted DNA was purified using RNase digestion, phenol-chloroform extraction, and isopropanol precipitation (Sambrook and Russell, 2001) and stored in 50 μ L of 10 mM Tris, 1 mM ethylenediaminetetraacetic acid at -20°C .

Each 25- μ L polymerase chain reaction (PCR) consisted of 20 ng template DNA, 2 mM MgCl_2 , 10 $\times$ reaction buffer, 0.2 mM mixed deoxyribonucleotides, 50 pM of a decanucleotide primer, and 0.5 unit KlenTaq (DNA Polymerase Technology, St. Louis, MO). A Techne PHC-3 thermal cycler (Bibby Scientific, Burlington, NJ) was used for all PCR amplifications. The thermal cycling parameters were derived from a related research study (Rodriguez et al., 1999). After an initial denaturation step of two cycles at 91 $^{\circ}\text{C}$ for 60 s, 42 $^{\circ}\text{C}$ for 15 s, and 72 $^{\circ}\text{C}$ for 70 s, 38 cycles were performed at 91 $^{\circ}\text{C}$ for 15 s, 42 $^{\circ}\text{C}$ for 20 s, and 72 $^{\circ}\text{C}$ for 20 s with a final extension step of 72 $^{\circ}\text{C}$ for 4 min.

Twenty-five primers (Operon Technologies, Alameda, CA) as used by Rodriguez et al. (1999) were screened to identify those primers that were both highly polymorphic and produced bright and distinct bands. Nine primers, OPR12, OPAO18,

OPAX1, OPAU3, OPAK10, OPAB4, OPAB19, OPK16, and OPAI2, were selected. DNA from the *C. chinense* accession TT-40 was used as a positive control in all amplifications and subsequent gel electrophoresis runs. A negative control was also included as part of each amplification. The PCR products were tested for their repeatability over time, and only reproducible bands were scored.

Amplified samples (15 μ L), along with a 1-kb ladder (Invitrogen; Life Technologies Corp., Carlsbad, CA), were subjected to gel electrophoresis at 5 V/cm on a 1.2% agarose gel containing ethidium bromide. The DNA was visualized on an ultraviolet transilluminator (UVP, Upland, CA) and photo-documented using a gel documentation system (Imagestore 7500; UVP). The presence or absence of the reproducible RAPD markers generated for each accession was given a score of 1 or 0, respectively.

DATA ANALYSIS. A non-metric multidimensional scaling (MDS) plot using Dice's similarity index of all accessions was drawn with PAST Version 1.78 (Hammer et al., 2001). Cluster analysis was performed to determine the number of distinct clusters using statistical software (NCSS Version 07.1.19; NCSS, Kaysville, UT). The clusters were revealed on the MDS plot. Analysis of molecular variance (AMOVA) was carried out in ARLEQUIN Version 3.11 (Excoffier et al., 2005) to test the significance of differences between the phylogenetic clusters as well as geographic regions within Latin America and the Caribbean. The significance of phylogeographical groups (geographically distinct groups of accessions falling within a phylogenetic group) discerned within each phylogenetic cluster was also tested using AMOVA. Unweighted pair group method with arithmetic mean dendrograms were drawn based on the Fixation index values of geographic regions and phylogeographic groupings. The presence of unique RAPD markers was also noted for the geographic regions, phylogeographic groups, and phylogenetic clusters within *C. chinense*. POPGENE Version 1.31 (Yeh and Boyle, 1997) was used to calculate three indices of diversity (i.e., Nei's diversity index, Shannon's index, and percentage of polymorphic loci).

Results

The nine arbitrary primers used generated 138 reproducible, polymorphic bands (Fig. 1). The number of polymorphic bands generated varied from nine to 20 depending on the primer with an average of 15.2 bands per primer.

DIVERSITY OF *C. CHINENSE*. Both Nei's and Shannon's diversity indices for the geographical regions within Latin America and the Caribbean (Table 2) mirrored each other in order of magnitude, whereas percentage polymorphic loci showed minor deviations from the former two measures. In general, the diversity indices were higher for the Upper and Lower Amazon regions than for the Caribbean or Central American groups.

PHYLOGENETIC STRUCTURE WITHIN *C. CHINENSE*. The accessions of *C. chinense* fell into three phylogenetic clusters (i.e., A, B, and C) based on the MDS plot (Fig. 2) and cluster analysis (data not shown), which was supported by AMOVA ($P < 0.05$) (Table 3).

Accessions from the Upper Amazon, Central America, Lesser Antilles, and Trinidad and Tobago fell solely or predominantly

Table 1. Country of origin, source of material, identifiers, and selected characteristics of *Capsicum* species used in this study.

UWI accession no. ^z	Country of origin	Source ^y	Germplasm identifiers/ other names	Morphological characteristics ^x						Phylogenetic group membership
				Flowers (no./axil)	Corolla color	Flower position	Calyx constriction	annular	Leaf pubescence	
1	CAC-89	Costa Rica	AVRDC	3	2	5	1	1	3	B
2	CAB-110	Belize	AVRDC	2	2	5	1	1	3	B
3	CAM-111	Mexico	AVRDC	3	2	5	1	1	3	B
4	CAM-112	Mexico	AVRDC	2	2	3	1	1	3	B
5	CAM-113	Mexico	AVRDC	3	2	3	1	1	3	B
6	CAB-220	Belize	USDA	3	2	3	1	1	3	B
7	CAC-224	Costa Rica	USDA	2	2	3	1	1	3	B
8	CAC-226	Costa Rica	USDA	3	2	3	1	1	3	B
9	CAC-227	Costa Rica	USDA	1	2	7	2	2	3	B
10	CAC-229	Costa Rica	USDA	3	2	3	1	1	7	B
11	CAM-294	Mexico	USDA	3	2	5	1	1	3	C
12	FGY-332	French Guiana	Field	3	2	3	1	1	3	C
13	FGY-333	French Guiana	Field	3	2	3	1	1	3	C
14	FGY-334	French Guiana	Field	3	2	3	1	1	3	C
15	FGY-335	French Guiana	Field	3	2	3	1	1	3	C
16	GAB-339	Bahamas	MOAB	1	2	7	2	2	5	In
17	GAB-340	Bahamas	MOAB	1	2	7	2	2	5	In
18	GAB-341	Bahamas	MOAB	1	2	7	2	2	5	In
19	GAB-342	Bahamas	MOAB	1	2	7	2	2	3	In
20	GAB-343	Bahamas	MOAB	1	2	7	2	2	7	In
21	GAB-344	Bahamas	MOAB	1	2	7	2	2	7	In
22	GAC-204	Cuba	IIHLD	3	2	5	1	1	3	A
23	GAJ-7	Jamaica	MOAJ	3	2	5	1	1	3	A
24	GAJ-129	Jamaica	MOAJ	3	2	3	1	1	7	A
25	GAP-149	Puerto Rico	AVRDC	2	2	5	1	1	3	A
26	GAP-213	Puerto Rico	USDA	3	2	3	1	1	3	A
27	GAP-214	Puerto Rico	USDA	3	2	3	1	1	3	A
28	GAP-215	Puerto Rico	USDA	3	2	3	1	1	3	A
29	GAP-218	Puerto Rico	USDA	3	2	5	1	1	3	A
30	GY-3	Guyana	CARDI	3	2	5	1	1	3	B
31	GY-8	Guyana	CARDI	3	2	5	1	1	3	B
32	GY-9	Guyana	CARDI	3	2	5	1	1	3	B
33	GY-172	Guyana	CARDI	3	2	3	1	1	3	B
34	GY-278	Guyana	CARDI	3	2	3	1	1	3	B
35	GY-280	Guyana	USDA	3	2	3	1	1	3	B
36	GY-281	Guyana	USDA	2	2	3	1	1	3	B
37	GY-282	Guyana	USDA	2	2	7	2	2	3	B
38	GY-283	Guyana	USDA	2	2	5	2	2	3	B
39	GY-284	Guyana	CARDI	3	2	3	1	1	3	B
40	GY-285	Guyana	CARDI	3	2	3	1	1	3	B
41	GY-286	Guyana	CARDI	3	2	3	1	1	3	B

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Table 1. Continued.

UWI accession no. ^z	Country of origin	Source ^y	Germplasm identifiers/ other names	Morphological characteristics ^x							Phylogenetic group membership
				Flowers (no./axil)	Corolla color	Flower position	Calyx annular constriction	Leaf pubescence			
42	Guyana	CARDI	CAPSBAR99094	3	2	3	1	3	B		
43	Guyana	CARDI	CAPSBAR99114	3	2	3	1	3	B		
44	Barbados	CARDI	'Edgehill yellow'	3	1	7	1	3	B		
45	Barbados	CARDI	'Barbados seasoning'	3	2	3	1	3	B		
46	Barbados	CARDI	'Local brown'	3	2	5	1	3	B		
47	Barbados	CARDI	'Barbados red'	3	1	3	1	3	B		
48	Barbados	CARDI	'CARDI purple'	3	2	3	1	3	B		
49	Barbados	CARDI	'CARDI Red'	3	2	5	1	3	B		
50	Guadeloupe	CARDI	'Sudan'	3	2	5	1	3	B		
51	Guadeloupe	CARDI	'Mauce'	3	2	5	1	3	B		
52	Guadeloupe	CARDI	'Poitout'	3	2	3	1	3	B		
53	Guadeloupe	CARDI	'Bazouka'	3	2	3	1	3	B		
54	Guadeloupe	CARDI	'INRA Touvin'	2	2	5	1	3	B		
55	Guadeloupe	Field	—	3	2	5	1	3	B		
56	Guadeloupe	Field	—	3	2	3	1	3	B		
57	St. Lucia	CARDI	'Red Flat'	3	2	5	1	3	B		
58	St. Lucia	Field	—	3	2	3	1	3	B		
59	St. Lucia	Field	—	3	2	3	1	3	B		
60	St. Lucia	Field	—	3	2	3	1	3	B		
61	St. Lucia	Field	—	3	2	3	1	3	B		
62	St. Lucia	Field	—	3	2	5	1	3	B		
63	Brazil	AVRDC	C04714	3	2	5	1	3	B		
64	Brazil	AVRDC	C04696	3	2	5	1	3	B		
65	Brazil	AVRDC	C05841	3	2	3	1	3	B		
66	Brazil	USDA	PI441633	2	2	5	1	3	B		
67	Brazil	USDA	PI497982	2	2	7	1	5	B		
68	Brazil	USDA	PI152452	2	2	7	1	3	B		
69	Brazil	USDA	PI441607	2	2	5	1	3	B		
70	Brazil	USDA	PI441613	2	2	3	1	3	B		
71	Brazil	USDA	PI438648	3	2	3	1	3	C		
72	Brazil	USDA	PI441599	3	2	3	1	3	C		
73	Brazil	USDA	PI441600	3	2	3	1	3	C		
74	Brazil	USDA	PI441601	3	2	3	1	3	C		
75	Brazil	USDA	PI441604	3	3	5	1	3	C		
76	Brazil	USDA	PI441605	3	2	3	1	3	C		
77	Brazil	USDA	PI441606	3	2	3	1	3	C		
78	Brazil	USDA	PI441608	2	2	3	1	3	C		
79	Brazil	USDA	PI441612	3	2	3	1	3	C		
80	Brazil	USDA	PI441616	3	2	3	1	3	C		
81	Brazil	USDA	PI441617	2	2	3	1	3	C		
82	Brazil	USDA	PI441618	3	2	3	1	3	C		

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Table 1. Continued.

	UWI accession no. ^z	Country of origin	Source ^y	Germplasm identifiers/ other names	Morphological characteristics ^x						Phylogenetic group membership
					Flowers (no./axil)	Corolla color	Flower position	Calyx annular constriction	Leaf pubescence		
83	LAM-309	Brazil	USDA	PI441623	3	2	7	1	3	C	
84	LAM-310	Brazil	USDA	PI441625	3	2	5	1	3	C	
85	LAM-311	Brazil	USDA	PI441626	2	2	3	1	3	C	
86	LAM-312	Brazil	USDA	PI441627	3	2	3	1	3	C	
87	LAM-313	Brazil	USDA	PI441631	2	2	3	1	3	C	
88	LAM-315	Brazil	USDA	PI497975	3	2	7	1	3	C	
89	LAM-316	Brazil	USDA	PI497977	3	2	5	1	5	C	
90	LAM-317	Brazil	USDA	PI497980	3	2	5	1	5	C	
91	SUR-202	Suriname	C.Chem	'Guyana red'	3	2	3	1	3	B	
92	SUR-237	Suriname	USDA	PI281428	3	2	5	1	3	B	
93	SUR-238	Suriname	USDA	PI439473	3	2	5	1	3	B	
94	SUR-324	Suriname	Field	GWG 3/'Madame Chaude'	2	2	5	1	3	C	
95	SUR-325	Suriname	Field	GWG 4	3	2	3	1	3	C	
96	TO-135	Tobago	Field	'Tobago 2'	3	2	3	1	3	B	
97	TO-143	Tobago	Field	'Faria yellow'	3	2	3	1	3	B	
98	TO-145	Tobago	Field	'Tobago 1'	3	2	5	1	3	B	
99	TO-182	Tobago	Field	'Tobago local dark green'	3	2	3	1	3	B	
100	TO-183	Tobago	Field	'Tobago local yellow'	3	2	3	1	3	B	
101	TO-184	Tobago	Field	'Tobago local brown'	3	2	3	1	3	B	
102	TT-11	Trinidad	Field	'Las Lomas'	3	2	3	1	3	B	
103	TT-12	Trinidad	Field	'Felicity 2'	3	2	3	1	3	B	
104	TT-13	Trinidad	Field	'Caroni cherry pepper'	3	2	3	1	3	B	
105	TT-14	Trinidad	Field	'El Dorado'	3	2	5	1	3	B	
106	TT-16	Trinidad	Field	'Seven pod'	3	2	3	1	5	B	
107	TT-17	Trinidad	Field	'Radoo red'	3	2	3	1	3	B	
108	TT-18	Trinidad	Field	'Radoo yellow'	3	2	3	1	3	B	
109	TT-19	Trinidad	Field	'Deo red1'	3	2	3	1	3	B	
110	TT-21	Trinidad	Field	'Deo green'	2	2	5	1	3	B	
111	TT-22	Trinidad	Field	'Deo yellow'	3	2	5	1	3	B	
112	TT-23	Trinidad	Field	'Deo yellow2'	3	2	3	1	3	B	
113	TT-26	Trinidad	Field	'Valencia 1'	3	2	3	1	3	B	
114	TT-28	Trinidad	Field	'Sampath 2'	2	2	3	1	3	B	
115	TT-29	Trinidad	Field	'Sampath 3'	3	2	5	1	3	B	
116	TT-34	Trinidad	Field	'Balandra basket'	2	2	3	1	3	B	
117	TT-35	Trinidad	Field	'Hot Pimento'	3	2	3	1	3	B	
118	TT-36	Trinidad	Field	'Toco red 1'	3	2	3	1	3	B	
119	TT-37	Trinidad	Field	'Toco yellow 2'	3	2	3	1	3	B	
120	TT-38	Trinidad	Field	'Toco red 2'	3	2	3	1	3	B	
121	TT-39	Trinidad	Field	'Davekas long pods 1'	3	2	5	1	3	B	
122	TT-40	Trinidad	Field	'Davekas short pods 2'	3	2	3	1	3	B	
123	TT-41	Trinidad	Field	'Peter 1'	3	2	3	1	3	B	

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Table 1. Continued.

UWI accession no. ^z	Country of origin	Source ^v	Germplasm identifiers/ other names	Morphological characteristics ^x						Phylogenetic group membership
				Flowers (no./axil)	Corolla color	Flower position	Calyx annular constriction	Leaf pubescence		
TT-42	Trinidad	Field	'Peter 2'	3	2	5	1	3	B	
TT-43	Trinidad	Field	'Cherry pepper'	3	2	3	1	3	B	
TT-44	Trinidad	Field	'Cumana 1'	3	2	3	1	3	B	
TT-46	Trinidad	Field	'Ishwar 3'	2	2	5	1	3	B	
TT-47	Trinidad	Field	'Bisram red'	3	2	3	1	3	B	
TT-48	Trinidad	Field	'Bisram yellow'	3	2	3	1	3	B	
TT-50	Trinidad	Field	'Angela 2'	2	2	5	1	3	B	
TT-52	Trinidad	Field	'Angela 4'	3	2	3	1	3	B	
TT-53	Trinidad	Field	'Seepersad 1'	3	2	5	1	3	B	
TT-54	Trinidad	Field	'Seepersad 2'	3	2	3	1	3	B	
TT-56	Trinidad	Field	'Victoria 1'	3	2	3	1	3	B	
TT-58	Trinidad	Field	'Victoria 3'	3	2	3	1	3	B	
TT-59	Trinidad	Field	'Victoria 4'	3	2	3	1	3	B	
TT-60	Trinidad	Field	'Victoria 5'	3	2	3	1	3	B	
TT-63	Trinidad	Field	'Paramin hot pepper'	3	2	3	1	3	B	
TT-64	Trinidad	Field	'Paramin seasoning'	3	2	3	1	3	B	
TT-65	Trinidad	Field	'Paramin yellow hot pepper'	3	2	3	1	3	B	
TT-66	Trinidad	Field	'Valencia pimento'	3	2	5	1	3	B	
TT-68	Trinidad	Field	'Penal pimento'	3	2	3	1	3	B	
TT-69	Trinidad	Field	'Penal congo pepper'	3	2	3	1	3	B	
TT-70	Trinidad	Field	'Princess congo pepper'	2	2	3	1	3	B	
TT-71	Trinidad	Field	'Seven pot'	3	2	3	1	5	B	
TT-74	Trinidad	Field	'Parbati 3'	3	2	3	1	3	B	
TT-75	Trinidad	Field	'Parbati 4'	2	2	3	1	3	B	
TT-76	Trinidad	Field	'Sieudass 1'	2	2	5	1	3	B	
TT-77	Trinidad	Field	'Sieudass 2'	3	2	5	1	3	B	
TT-78	Trinidad	Field	'Lal pepper'	3	2	5	1	3	B	
TT-79	Trinidad	Field	'Maracas'	3	2	3	1	3	B	
TT-83	Trinidad	Field	'Big sun'	3	2	3	1	3	B	
TT-84	Trinidad	Field	'Rio habanero'	3	2	3	1	5	B	
TT-85	Trinidad	Field	'Isaac local'	3	2	3	1	3	B	
TT-95	Trinidad	Field	'Local white yellow skin'	3	2	3	1	3	C	
TT-99	Trinidad	Field	'Congo pepper 5'	3	2	5	1	3	B	
TT-100	Trinidad	Field	'Balram red'	3	2	3	1	3	B	
TT-101	Trinidad	Field	'Scorpion pepper (Red)'	3	2	3	1	3	B	
TT-102	Trinidad	Field	'Mycoc yellow'	3	2	3	1	3	B	
TT-105	Trinidad	Field	'Balram yellow'	3	2	3	1	3	B	
TT-106	Trinidad	Field	'Yellow hot'	3	2	3	1	3	B	
TT-131	Trinidad	Field	'Tabaquite 3'	3	2	3	1	3	B	
TT-133	Trinidad	Field	'Faria red'	3	2	3	1	3	B	
TT-134	Trinidad	Field	'Ishwar 1'	3	2	7	1	3	C	

continued next page

Table 1. Continued.

UWI accession no. ^z	Country of origin	Source ^y	Germplasm identifiers/ other names	Morphological characteristics ^x						Phylogenetic group membership
				Flowers (no./axil)	Corolla color	Flower position	Calyx annular constriction	Leaf pubescence		
165	Trinidad	Field	'Gasparillo 1'	3	2	3	1	3	B	
166	Trinidad	Field	'Gasparillo 3'	3	2	3	1	3	B	
167	Trinidad	Field	'Gasparillo 4'	3	2	3	1	3	B	
168	Trinidad	Field	'Mixed Gasparillo red smooth'	3	2	5	1	3	B	
169	Trinidad	Field		—	3	2	3	1	5	B
170	Trinidad	Field		—	3	2	3	1	3	B
171	Trinidad	Field		'Congo'	3	2	3	1	3	B
172	Trinidad	Field	'Marlon'	3	2	5	1	3	B	
173	Trinidad	Field	'Mixed Gasparillo red rough'	3	2	3	1	3	B	
174	Trinidad	Field	'Jermaine'	3	2	3	1	3	B	
175	Bolivia	AVRDC	C04533	3	2	5	1	5	B	
176	Bolivia	USDA	PI238050	3	2	3	1	3	B	
177	Bolivia	USDA	PI281303	2	2	5	1	3	In	
178	Bolivia	USDA	PI543182	3	2	5	1	3	B	
179	Bolivia	USDA	PI213918	3	2	3	2	3	In	
180	Bolivia	USDA	PI260485	3	2	5	1	5	B	
181	Bolivia	USDA	PI543208	3	2	3	1	3	B	
182	Colombia	USDA	Gri9308	1	2	5	1	3	B	
183	Colombia	USDA	PI257091	1	2	3	2	3	In	
184	Colombia	USDA	PI290972	3	2	3	1	3	B	
185	Ecuador	AVRDC	C04539	2	2	5	1	5	B	
186	Ecuador	USDA	PI355820	1	2	5	0	3	B	
187	Ecuador	USDA	PI257136	3	2	3	1	3	B	
188	Ecuador	USDA	PI585252	3	2	3	1	3	B	
189	Peru	AVRDC	C04477	3	2	3	1	3	B	
190	Peru	AVRDC	C02677	1	2	5	1	3	B	
191	Peru	USDA	PI215731	3	2	5	1	3	B	
192	Peru	USDA	PI260501	3	2	7	1	3	B	
193	Peru	USDA	PI260509	3	2	3	1	3	In	
194	Peru	USDA	PI315015	2	2	3	1	3	B	
195	Peru	USDA	PI315019	2	2	7	1	3	B	
196	Peru	USDA	PI315023	2	2	3	1	3	B	
197	Peru	USDA	PI315031	2	2	3	1	5	B	
198	Peru	USDA	PI315028	3	2	3	1	3	B	
199	Venezuela	USDA	PI281441	3	2	3	1	3	B	
200	Venezuela	USDA	PI439482	3	2	3	1	3	B	
201	Venezuela	USDA	PI439486	3	2	3	1	3	B	
202	Brazil	AVRDC	C05635	2	2	7	0	5	B	
203	Sri Lanka	AVRDC	C05645	2	2	7	0	5	B	
204	breeding material	AVRDC	PBC0635	2	2	7	0	5	B	
205	India	AVRDC	C0223	1	1	3	0	5	B	

continued next page

Table 1. Continued.

UWI accession no. ^z	Country of origin	Source ^y	Morphological characteristics ^x							Phylogenetic group membership
			Germplasm identifiers/ other names	Flowers (no./axil)	Corolla color	Flower position	Calyx annular constriction	Leaf pubescence		
206	India	AVRDC	C00266	3	1	3	0	3	B	
207	USA	AVRDC	C00268	3	1	7	0	3	B	

^x*“Capsicum”* accessions housed in the University of the West Indies, St. Augustine, Trinidad (UWI) collection: *C. chinense* (1–201), *C. baccatum* (202–204), and *C. annuum* (205–207), each with a unique numeric identifier.

^yDonating agencies include Asian Vegetable Research Development Center, Tainan, Taiwan (AVRDC); U.S. Department of Agriculture, Beltsville, MD (USDA); Ministry of Agriculture, Nassau, Bahamas (MOAB); Ministry of Agriculture, Kingston, Jamaica (MOAJ); Caribbean Agricultural Research and Development Institute, St. Augustine, Trinidad (CARDI); Instituto de Investigaciones de Hortalizas ‘Liliana Dimitrova’ of Cuba, Artemisa, Cuba (IHLD); Caribbean Chemicals & Agencies Ltd., El Socorro, Trinidad (C. Chem). “Field” refers to samples collected in farmers’ fields in the stipulated country and held in the UWI collection.

^zFlowers per axil (1 = one flower, 2 = two flowers, 3 = more than two flowers), corolla color (1 = white, 3 = not white), flower position (3 = pendant, 5 = intermediate, 7 = erect), calyx annular constriction (0 = absence, 1 = presence, 2 = indeterminate), leaf pubescence (3 = sparse, 5 = medium, 7 = dense). Phylogenetic cluster membership (A–C) of individual accessions as determined through cluster analysis and diagrammed in Figure 2.

In = uncertain membership.

into Cluster B, whereas accessions from the Lower Amazon fell predominantly (71%) into Cluster C and the remainder (29%) in Cluster B. The situation was reversed in the Guianas including Venezuela, where most accessions (77%) fell into Cluster B with a minority falling (23%) into Group C. Accessions from the Greater Antilles fell into a unique Cluster A (60%) with the remainder falling into an outgroup.

DIVERSITY WITHIN THE PHYLOGEOGRAPHIC GROUPS. Accessions from the geographic regions that fell into the three phylogenetic clusters (A, B, and C) formed nine subgroups herein referred to as phylogeographic groups (Table 4). The most diverse phylogeographic group with regard to all three diversity indices was the Lower Amazon accessions that fell into phylogenetic Cluster C followed by the Upper Amazon accessions that fell into phylogenetic Cluster B. Among those regions belonging to phylogenetic Cluster B, the greatest diversity estimated by the indices was in the Upper Amazon followed by Central America, Guianas including Venezuela, Trinidad and Tobago, and Lesser Antilles in that order.

STRUCTURE WITHIN PHYLOGEOGRAPHIC GROUPS WITHIN PHYLOGENETIC CLUSTERS. An AMOVA conducted between phylogeographic groups within each phylogenetic cluster indicated that there was some underlying structure within phylogenetic Clusters B and C (data not shown) at ($P < 0.05$). However, the structure was relatively weak, suggested by the low percentage (8%) of the total variation being attributed to between-population variation.

PHYLOGENETIC RELATIONSHIP AMONG REGIONS AND GROUPS. The dendrogram (Fig. 3A) generated for the various geographic regions indicated that accessions from Central America, Guianas including Venezuela, Upper Amazon, Trinidad and Tobago, and Lesser Antilles were more similar among each other than to Lower Amazon or to the Greater Antilles. The dendrogram of phylogeographic groups (Fig. 3B) was similar to that generated for geographic regions. The phylogeographic groups belonging to Cluster A (Greater Antilles) were more similar to Cluster C (Lower Amazon and a few accessions from the Guianas) than those belonging to Cluster B (Central America, Guianas including Venezuela, Upper Amazon, Trinidad and Tobago, and Lesser Antilles).

MOLECULAR CHARACTERIZATION OF THE PHYLOGENETIC CLUSTERS. From 11 discriminatory markers (RAPD bands) accounting for the variation among the three phylogenetic groups, eight markers were monomorphic in phylogenetic Cluster A, two in Cluster C, and one in Cluster B (data not presented).

Discussion

THE PHYLOGENETIC STRUCTURE OF *C. CHINENSE*. This study for the first time recognized three phylogenetic clusters within *C. chinense* in the Latin America and the Caribbean regions. Two of the Clusters (B and C) were widespread throughout this region, whereas one (Cluster A) was specific to the Greater Antilles region of the Caribbean. Cluster B, which had its greatest diversity in the Upper Amazon region according to diversity estimates (Tables 2 and 4), was the most geographically diverse and predominant group, extending from Central America in the north to the Guianas, Venezuela, Trinidad, and the Lesser Antilles to the east. There was also evidence of substructures within Clusters B and C based on geography (phylogeographic groups), but the substructures were weak and accounted for only 8% of the total variation, indicating that

sufficient geographical isolation may not have existed post-domestication of *C. chinense* in the Americas.

Similar clustering based on geography was observed in the genetic structure of *C. baccatum*, a pepper species believed to have been domesticated 4000 years ago in the Andean region at the same time as *C. chinense* (Pickersgill, 2007). There are two genetic groups identified for the domesticated *C. baccatum*; i.e., those from the western region of South America consisting of accessions from Peru, Columbia, Ecuador, Bolivia, Chile, and Argentina and those from the eastern areas of Paraguay and eastern Argentina (Albrecht et al., 2011). *C. annuum*, a domesticate that is believed to have originated in the Mesoamerican region 6000 years ago according to archeological evidence (Perry et al., 2007), has also been shown to have a geographical structure to its diversity. For this species, three geographic patterns of genetic differentiation are observed: eastern Mexico, western Mexico, and the Yucatan Peninsula (Aguilar-Melendez et al., 2009).

Within each geographical region for *C. chinense*, there were often accessions from more than one phylogenetic cluster. For instance, the majority of accessions from the Lower Amazon region fell into phylogenetic Cluster C and some accessions fell into phylogenetic Cluster B. This could have been an artifact resulting from bird dispersal (Tewksbury and Nabhan, 2001) or anthropogenic effects, because *Capsicum* is a highly traded

commodity. To eliminate the effects of humans and/or bird dispersal, genetic diversity indices were calculated separately for each phylogeographic group (Table 4). The results indicate that the Lower Amazon accessions belonging to phylogenetic Cluster C and Upper Amazon accessions belonging to phylogenetic Cluster B had the greatest diversity estimates among the nine phylogeographical groups investigated. The fact that phylogenetic Cluster B, typical of the Upper Amazon, is the most widespread and the most variable in Latin America and the Caribbean supports previous reports (Eshbaugh, 1993; McLeod et al., 1982) that the Upper Amazon is probably the center of domestication of *C. chinense*.

The clear distinction between the Upper Amazon B and the Lower Amazon C, from continental South America, suggests that the dense Amazonian forest between these regions may have served as an effective barrier of panmixis. A similar divergence between cocoa populations from the Upper Amazon and Lower Amazon has also been reported (Motamayor et al., 2008).

DISPERSION AND THE LIKELY ORIGIN OF *C. CHINENSE* PHYLOGENETIC GROUPS. In *C. annuum* and *C. baccatum*, there is more genetic diversity in the wild forms than in the domesticates (Aguilar-Melendez et al., 2009; Albrecht et al., 2011) and this has been used to identify putative centers of diversity and domestication events. However, it is difficult to determine the wild forms of *C. chinense* as clearly as in the other domesticates (Eshbaugh, 1993) and there is believed to be no true form of wild *C. chinense* (Walsh and Hoot, 2001). However, by examining the history of the Latin America and the Caribbean region as revealed by archaeological studies, in conjunction with the data obtained in this and other studies, one can postulate possible dispersal paths for *C. chinense* within the Caribbean region.

Earliest archaeological records point to the existence of remains of *C. annuum* in Central America more than 6000 years ago and *C. chinense* in Peru $\approx$ 4000 years ago (Perry et al., 2007), which supports the notion that *C. chinense* was domesticated in South America (Eshbaugh, 1993; McLeod et al., 1982; Pickersgill, 1971).

From historical descriptions, it is clear that Amerindians grew *C. chinense* in the Caribbean (Pickersgill, 2005; Rouse, 1993).

Fig. 1. Gel image showing random amplification of polymorphic DNA (RAPD) fragments amplified by Operon Primer OPR12 (Operon Technologies, Alameda, CA) in 14 *Capsicum chinense* accessions (lanes 1–8 from the Upper Amazon, lanes 9–14 from the Lower Amazon region) on a 1.2% agarose gel with 1-kb ladder (far left lane). The arrows represent fragment sizes, and letters A–F represent polymorphic (absence or presence) RAPD bands for the 14 accessions.

Table 2. Comparison of three diversity indices of *Capsicum chinense* within geographical regions in Latin America (Lower Amazon, Upper Amazon, Guianas including Venezuela, Central America) and the Caribbean (Greater Antilles, Lesser Antilles and Trinidad and Tobago).

Regions	Accessions (no.)	Nei's index	Shannon's index	Polymorphic loci (%)
Latin America	89	0.278	0.423	95
Lower Amazon	28	0.280 (1) ^z	0.419 (1)	82 (1)
Upper Amazon	24	0.218 (3)	0.330 (3)	70 (2)
Guianas/Venezuela	26	0.208 (4)	0.316 (4)	68 (3)
Central America	11	0.192 (5)	0.288 (5)	56 (6)
Caribbean region	112	0.218	0.343	88
Greater Antilles	14	0.257 (2)	0.375 (2)	66 (4)
Trinidad and Tobago	79	0.156 (6)	0.246 (6)	63 (5)
Lesser Antilles	19	0.152 (7)	0.227 (7)	44 (7)
Total accessions	201			

^zThe numbers in parentheses refer to the numerical ranking of diversity in descending order.

Fig. 2. Non-metric multidimensional scaling plot (MDS) showing the distinct clustering (Dice similarity index) of *Capsicum chinense* accessions from seven geographic regions (*Guianas including Venezuela, Δ Lesser Antilles, + Central America, □ Greater Antilles, Upper Amazon, ▼ Lower Amazon, ■ Trinidad and Tobago) into three phylogenetic clusters, A, B, and C. *Capsicum annuum* and *Capsicum baccatum* accessions are indicated within the dotted lines.

Table 3. Analysis of molecular variation between three phylogenetic Clusters A, B, and C of *Capsicum chinense*.

Source of variation	df	Sum of squares	Variance components	Variation (% of total)	P
Among populations	2	317.502	4.79540	32.70	<0.05
Within populations	188	1855.137	9.86775	67.30	<0.05
Total	190	2172.639	14.66315		

Table 4. Genetic diversity estimates of *Capsicum chinense* within nine phylogeographical groups based on three diversity indices.

Phylogeographic groupings	Accessions (% of total)	Nei's index	Shannon's index	Polymorphic loci (%)
Upper Amazon B ^z	88	0.186 (2) ^y	0.282 (2)	61 (3)
Guianas B	77	0.170 (4)	0.262 (4)	59 (4)
Trinidad and Tobago B	97	0.157 (6)	0.248 (5)	63 (2)
Lesser Antilles B	100	0.152 (7)	0.227 (7)	44 (6)
Central America B	82	0.176 (3)	0.262 (3)	49 (5)
Lower Amazon B	29	0.147 (8)	0.218 (8)	41 (8)
Lower Amazon C	71	0.272 (1)	0.406 (1)	78 (1)
Guianas C	23	0.131 (9)	0.194 (9)	36 (9)
Greater Antilles A	60	0.164 (5)	0.241 (6)	44 (7)

^zA, B, and C denote phylogenetic clusters of *Capsicum chinense*.

^yThe numbers in parentheses refer to the descending numerical order of diversity.

There are two plausible explanations for the presence of *C. chinense*, domesticated in the Upper Amazon, in the Antillean islands and Central America: 1) the dispersal of seeds by birds (Tewksbury and Nabhan, 2001) favored by south

to north wind currents (Reid, 2009); or 2) deliberate transportation of the crop by the ancient migrating Amerindians.

Launching from the banks of the Orinoco River, it is suggested that Amerindians from South America first settled

Fig. 3. Phylogenetic relationship (A) between *Capsicum chinense* from different geographic regions and (B) between different phylogeographic groupings (A–C) based on unweighted pair group method with arithmetic mean (UPGMA) of Fixation index (Fst) values. The geographic regions included Lower Amazon (LAM); Greater Antilles (GA); Upper Amazon (UA); Guianas including Venezuela (GV), Central America (CA), Lesser Antilles (LA) Trinidad and Tobago (TT). Phylogenetic clusters (A–C) as described in Fig. 2. Bootstrap values based on 1000 replicates.

in Trinidad $\approx$ 5000 BCE to 200 BCE and from there moved up the Antilles islands. Another group of Amerindians, the Casimiroids, were believed to have emerged from Belize 4000 BCE to 400 BCE (Mesoamerica) and moved into Cuba and Hispaniola (Reid, 2009). The human migratory model therefore suggests a possible two-pronged entry into the island chain: one from South America into Trinidad and Tobago and a northward migration into the Lesser Antilles and the other from Central America through the Yucatán to the Greater Antilles.

The molecular data derived in this experiment suggest that there may have been three evolutionary directions taken by the species resulting in the three phylogenetic Clusters A, B, and C.

Phylogenetic Cluster B, the largest and most widespread phylogenetic group, contains accessions from the Upper Amazon, Guianas including Venezuela (77% of accessions), Trinidad and Tobago, Lesser Antilles, and Central America.

Furthermore, the diversity measures for the phylogeographic groups within Cluster B indicate that the samples from the Upper Amazon were the most diverse followed by those in the Guianas including Venezuela, Trinidad and Tobago, and Lesser Antilles in that order. Frankham et al. (2002), based on a collation of many studies, showed that the diversity of species decreases away from the mainland. This supports a migratory route from the Upper Amazon through Guianas including Venezuela, into Trinidad and the Lesser Antilles islands. This migratory route is also supported by archaeological records of human migration as well as the avian migration model (Reid, 2009). The fact that phylogenetic Cluster B did not reach the Greater Antilles can also be explained by archaeological evidence that points to an Amerindian front resisting any migration from the Lesser Antilles into the Greater Antilles (Reid, 2009).

The significantly diminished variation of Central America B, compared with that for Upper Amazon B, suggests a second migratory route of *C. chinense* from the Upper Amazon to Central America. The Isthmus of Panama, a constriction, only 48 km at its narrowest point, which squeezes the American continent into South and Central America, is believed to have formed 3 million years ago (Duque-Caro, 1990). It has long been a matter of contention whether the Isthmus of Panama provided an efficient route of north–south dispersion of species between the mega centers, and if so, how effective a path it has been during the historical ages. Some (Myers, 1964) argue, that since the last recession of the cordilleran glaciers, the Isthmus has been covered by dense tropical forest and plagued by numerous tropical diseases creating an unwelcome environment for human habitation and hence an inefficient dispersal route. Others (Dickau et al., 2007) report evidence of dispersal of maize (*Zea mays*), manioc (*Manihot esculenta*), and arrowroot (*Maranta arundinacea*) through the Isthmus of Panama as early as 7000 CE. However, the significantly diminished diversity of *C. chinense* in Central America B suggests the Isthmus of Panama may have served as an inefficient dispersal path of *C. chinense* into Central America. Although other studies (Clement et al., 2010; Gepts and Bliss 1988; Vigouroux et al., 2008) postulate a path from Meso-America to South America through the Central American bridge for the Mesoamerican domestication complex [*C. annuum*, wild beans (*Phaseolus vulgaris*), and maize], this study and archeological studies favor domestication in the Upper Amazon and a dissemination path in the opposite direction for *C. chinense*.

The origin of phylogenetic Cluster A that is peculiar to Greater Antilles is not clear. One could postulate an origin through recent hybridization of *C. chinense* with other cultivated *Capsicum* species indigenous to Mesoamerica such as *C. annuum* or *C. frutescens* that coinhabit the region (Bosland and Votava, 2000). Alternatively, in the absence of gene flow from mainland populations, small island populations may have undergone considerable genetic drift.

The third migratory route for *C. chinense* may have occurred from the Upper Amazon to the Lower Amazon in the distant past. Evidence for this comes from the existence of two phylogenetic Clusters, B and C in the Lower Amazon of which Cluster C is the dominant one (80% of accessions). This suggests that these Lower Amazon accessions may have become isolated from the main gene pool of the Upper Amazon, which may have led to the evolution of phylogenetic Cluster C. As mentioned previously, the Amazonian forests may have

served as such a barrier that could have led to such genetic isolation. The presence of a few accessions typical of Cluster B may be a result of recent anthropogenic events. Alternatively, two separate domestication events may have founded the two populations.

IMPLICATIONS TO BREEDING. In this study, molecular markers were used to assess the genetic diversity within populations of *C. chinense* and to understand the genetic structure of populations. The presence of three phylogenetic clusters in the species indicates the potential of investigating interpopulation heterosis approaches in hybrid breeding. Furthermore, it is possible to concentrate complementary genes among the various phylogenetic clusters through reciprocal recurrent selection.

Molecular markers developed in this study will also assist in the effective management of collections of *C. chinense* by removing duplicates and establishing a core collection. In addition, molecular techniques aid the ability of researchers to determine the identity, purity, and stability of cultivars. This information can then be used to protect the rights of breeders and research institutions and to develop effective seed quality control programs (Ilbi, 2003). High-density genetic maps developed in the future can also allow the application of marker-assisted selection in *C. chinense*.

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