Introgression of Solanum chacoense (Solanum sect. Petota): Upland Populations Reexamined

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Introgression of *Solanum chacoense* (*Solanum* sect. *Petota*): Upland Populations Reexamined

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ABSTRACT. *Solanum chacoense* is a highly variable wild potato species widely distributed from central Bolivia to central Argentina, from sea level to over 3,000 m in the Andes. Upland populations of *S. chacoense* were thought to have arisen from introgression of lowland populations with *S. microdontum*. Our study uses morphological data, molecular data from single- to low-copy nuclear DNA restriction fragment length polymorphisms (RFLP's), and random amplified polymorphic DNA (RAPD's) to reinvestigate this hypothesis. The data do not support introgression but show relative equal divergence of upland and lowland populations from *S. microdontum*. These results suggest that other hybridization hypotheses need to be reexamined in sect. *Petota*. However, the similarity of many taxa in the group and alternative explanations of morphological or molecular intermediacy will complicate conclusions of hybridization.

Solanum L. sect. *Petota* Dumort., the potato and its wild relatives, includes 232 species (Hawkes 1990). An alternative classification (Child 1990), supported by morphological and chloroplast DNA (cpDNA) data (Spooner et al. 1993) removes the eight non-tuberous species to sect. *Etuberosum* (Bukasov and Kameraz) A. Child, sect. *Juglandifolium* (Rydb.) A. Child, and sect. *Lycopersicum* (Mill.) Wettst. The remaining 224 tuberous species occur from the southwestern United States to south central Chile, with the greatest diversity of species in Peru and Bolivia. Species occur from sea level to over 4,500 m, but most occur between 2,000 and 3,500 m. Most members of sect. *Petota* are diploids (2*n*=24), but a few are triploid, tetraploid, pentaploid, or hexaploid (Correll 1962; Hawkes 1990).

Section *Petota* is taxonomically complex. There is much disagreement regarding species boundaries, affiliation of species to series, rank of infraspecific taxa, and hypotheses of hybridization (Spooner and van den Berg 1992a). Diploid and polyploid hybrid speciation and introgressive hybridization are hypothesized to be common and important evolutionary mechanisms in sect. *Petota* (Ugent 1970; Hawkes 1978, 1990). Approximately 12% (26) of the 224 species in sect. *Petota* have been hypothesized to have arisen by hybrid speciation (listed in Spooner and van den Berg 1992b). In addition, local hybridization not leading to widespread introgression or speciation is believed to be common. Hawkes and Hjerting (1969, 1989) provided extensive lists of such putative occasional hybrids.

Some species in sect. *Petota* have strong interspe-

cific reproductive isolating mechanisms. One mechanism is governed by a process involving ratios of maternal/paternal effective ploidy in the endosperm, and evidenced by endosperm breakdown (Endosperm Balance Number, EBN; Hanneman 1994). In addition, some interspecific crosses within EBN levels are inhibited by stylar barriers (Abdalla and Hermsen 1972; Fritz and Hanneman 1989). However, most diploid species, especially those in South America, can artificially be hybridized to form viable interspecific progeny, although some may show reduced fertility in subsequent generations (Hawkes 1990). Often, different species occur sympatrically, providing the opportunity for natural hybridization (Hawkes 1978, 1990).

Solanum chacoense Bitter was the first wild potato species investigated for introgressive hybridization in sect. *Petota* (Hawkes 1962). The latest comprehensive taxonomic revision (Hawkes 1990) and a subsequent review of evolution in sect. *Petota* (Hawkes 1994) continue to cite *S. chacoense* as one of the better-documented examples of introgressive hybridization in the group. A recent review of introgression in plants, however, cites *S. chacoense* as an example of an introgression hypothesis where alternative explanations were not adequately pursued (Rieseberg and Wendel 1993).

Solanum chacoense grows from central Bolivia south to central Argentina, Brazil, and Paraguay, and from the Atlantic coast to the Andes, up to 3,700 m. It covers an area 1,700 km north-south by 1,500 km east-west (Hawkes 1962), and is one of the most widely distributed wild potato species.

Hawkes (1962) proposed that the widespread variation within *S. chacoense* was partitioned by altitude and latitude. He suggested that the species originally was confined to the low plains, but expanded its range to higher elevations in the Andes subsequent to hybridization with *S. microdontum* Bitter, a sympatric species in mid to high elevations in the Andes. *Solanum microdontum* also is highly variable, distributed from central Bolivia to central Argentina, from 1,000 m to 3,900 m (van den Berg and Spooner 1992). Hawkes proposed that this hybridization was a recent event, occurring after habitat disturbance by European colonists allowed the two species to come into sympatry. Both species are diploid obligate outcrossers, and artificial hybrids between them are highly fertile, at least in the F_1 generation (Brücher 1953; Hawkes and Hjerting 1969).

Hawkes supported his hypothesis by a morphological analysis (scatter diagrams and hybrid indices) of herbarium specimens of *S. chacoense*. He proposed *S. microdontum* as the introgressant parent (although he did not include this species in his numerical analysis) because *S. microdontum* has morphological features similar to mountain forms of *S. chacoense*: broader stem wings, shorter petioles, denser leaf pubescence, higher pedicel articulation, and longer calyx acumens (the acuminate tip of the calyx). Both Hawkes (1962) and Brücher (1966) suggested that more controlled studies of variation in common garden plots would provide more reliable data to this introgression hypothesis.

The purpose of our study is to reexamine the hypothesis of hybridization involving *S. chacoense* and *S. microdontum*. We measure the morphological characters used by Hawkes (1962), and additional morphological characters, from plants collected from throughout their range in Argentina and planted in a common garden. We also gather data from single- to low-copy nuclear restriction fragment length polymorphisms (RFLP's) and random amplified polymorphic DNA (RAPD's). These molecular characters have been shown to be useful for investigating hybridization (e.g., Crawford et al. 1993; Rieseberg 1995; Rieseberg and Ellstrand 1993).

MATERIALS AND METHODS

Morphological Analysis.

SPECIES.   A total of 66 accessions of *S. chacoense* and 11 accessions of *S. microdontum* were analyzed (Table 1). All accessions came from the National Research Support Program-6 (NRSP-6; Bamberg et al. 1996). Identifications have been provided in past years by visiting taxonomists during on-site visits to NRSP-6 to inspect living representatives in field plots (Hanneman 1989).

We mapped the accessions to 20 generalized areas (Fig. 1; Table 1). These areas represent the broadest total geographic range available from the NRSP-6 collection for *S. chacoense*, and the broadest Argentinian range of *S. microdontum*. Seeds were planted in a greenhouse in early May, 1993, seedlings were transferred to peat pots in late May, and six individuals per accession were transplanted together in rows in a field plot in Sturgeon Bay in early June.

DATA MEASUREMENT.   The middle four surviving plants per row were measured for each accession. We measured a total of 38 characters (Table 2) on each plant. Of these, 31 characters were quantitative and seven were qualitative. Measurements of leaves were made on the largest leaf per plant. Floral characters were taken from the uppermost inflorescence. The mean of these four plants was used as representative of the accession.

Corolla colors were measured with the aid of the R.H.S. Colour Charts (Royal Horticultural Society 1986), based on recommendations of Tucker et al. (1991), and scored as 0–4 from yellow (color 2D), to white (color 155D), to darkening shades of violet-blue (colors 112D, 92D, 84C).

DATA ANALYSIS.   The operational taxonomic unit (OTU) was the accession, using averaged data for each character. The averaged data were standardized (STAND) using the default options YBAR and STAND of NTSYS-pc®, version 1.70 (Rohlf 1992). A Principal Coordinates Analysis (PCO) of all accessions was performed from a distance matrix (DIST), followed by double centering (DCENTER) and eigenvectors (EIGEN). A Principal Components Analysis (PCA) of all accessions was performed from a correlation matrix (CORR) and eigenvectors (EIGEN).

PCO, PCA, and canonical variates analyses (CVA; SAS Institute Inc. 1988) were performed for the entire data set, and for two subsets of the data set as defined below. Stepwise Discriminant Analyses (SDA; SAS Institute Inc. 1988) additionally were performed for the same subsets of the *S. chacoense* data set. The first subset analyzed differences within *S. chacoense* by elevational ranges: 1) below 1,500 m, above 1,500 m;   2) 0-1,000 m, 1,001 m-1,500 m, 1,501 m-2,000 m, and 2,001 m, and above, and   3) 0–700 m, 701 m-1,000 m, 1,001
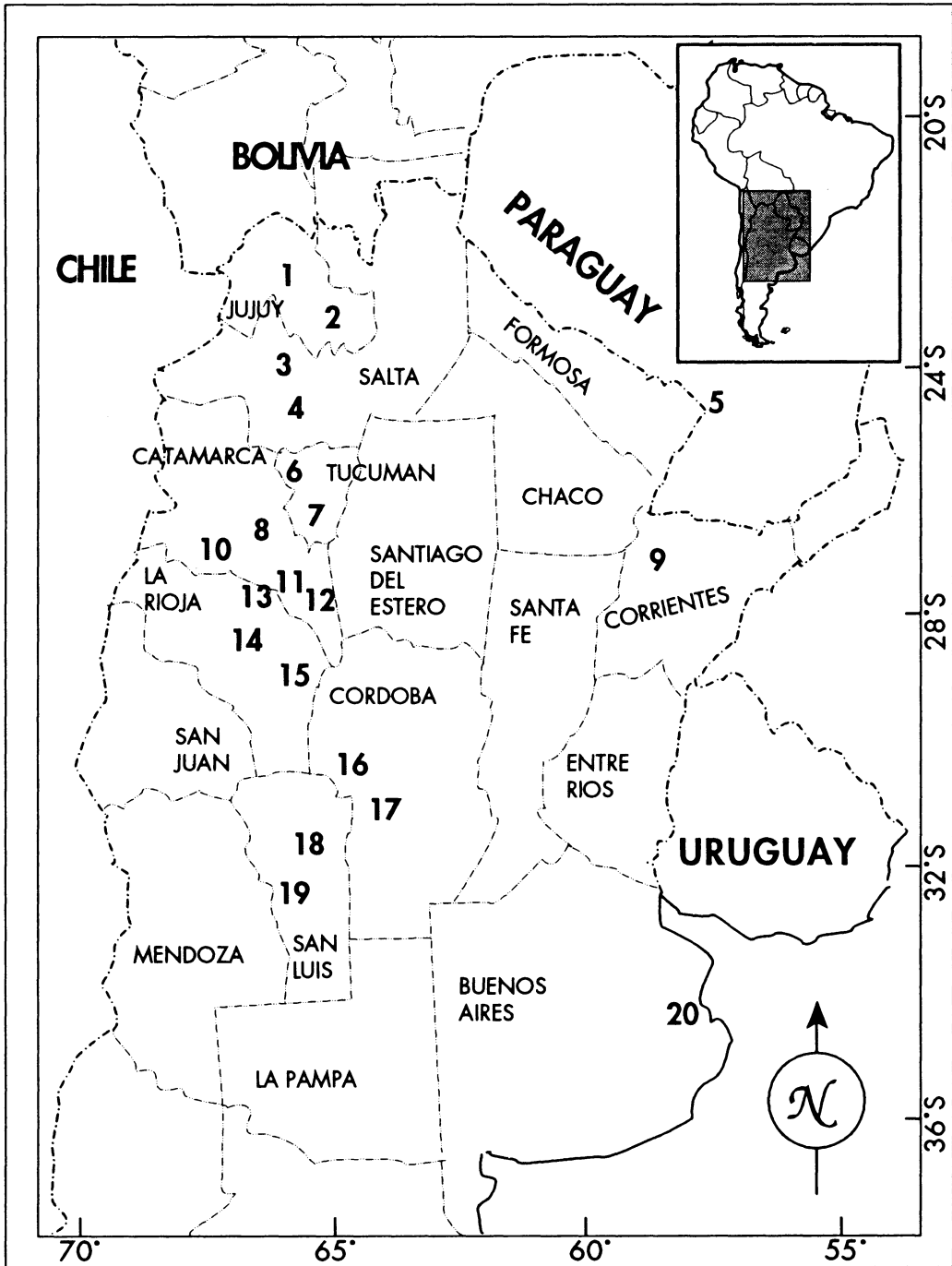
FIG. 1. Map of study area in southern South America (shaded area of inset map) showing the 20 generalized areas of *Solanum chacoense* and *S. microdontum* accessions examined in this study using morphology, RFLP's and RAPD's (see Table 1).

TABLE 1. Accessions of *Solanum* sect. *Petota* examined. Vouchers are deposited at the herbarium of the National Research Support Program, Sturgeon Bay, Wisconsin. [1]Taxon abbreviations follow Hawkes (1990): chc = *S. chacoense*, mcd = *S. microdontum*. [2]M = Accession used in the morphological study, D = Accession used in the molecular study, followed, in parentheses, by the number of individual plants examined in the RFLP and RAPD studies. [3]Generalized map areas, R = Accession used in the RAPD study, see Fig. 1. [4]United States Department of Agriculture Plant Introduction Numbers. [5]Collector unknown; accession from the Commonwealth Potato Collection, Scotland. [6]PI number not yet assigned. [7]Collector unknown; accession from the Erwin Baur Sortiment Genebank, Germany.

Taxcn[1]	Study[2]	Area[3]	PI[4]	Collector	Locality
1. chc	M	1	217451	*Sleumer 3566*	Argentina. Jujuy: Tumbaya, Volcán; 2,100 m; 24°43'S, 65°40'W.
2. chc	M	1	275136	*Hjerting 349*	Argentina. Jujuy: Tilcara, Quebrada de Yaquis Pampa, El Estanque, down from San Antonio; 2,300 m; 23°36'S, 65°08'W.
3. chc	M	1	458309	*Hoffman 1560*	Argentina. Jujuy: Capital, Encrucijada de El Chani; 2,450 m; 24°03'S, 65°28'W.
4. chc	M, D (3)	1	498316	*Hoffman 1554 × 1582*	Argentina. Jujuy: 1554 = Tumbaya, Saladillo, 24°34'S, 66°13'W. 1582 = Molinos; 3,700 m[?]; 24°43'S, 65°40'W.
5. chc	M, D (3), R (3)	1	500020	*Okada 7545*	Argentina. Jujuy: Tumbaya, near Volcán, 13 km N of León on Route 59; 2,000 m; 23°58'S, 65°27'W.
6. chc	M	2	472810	*Hoffman 1533*	Argentina. Jujuy: Santa Bárbara, Palma Sola; 750 m; 22°40'S, 64°18'W.
7. chc	M	3	189217	*CPC 51B*[5]	Argentina. Salta: near town of Salta, Quebrada de San Lorenzo; 1,500 m; 64°10'S, 24°20'W.
8. chc	M	3	414143	*Okada 4822*	Argentina. Salta: Rosario de Lerma, Virrey Toledo, Route 51, km 33.5; 1,600 m; 24°54'S, 65°39'W.
9. chc	M	3	458308	*Hoffman 1550*	Argentina. Salta: Rosario de Lerma, near Campo Quijno; 1,600 m; 24°53'S, 65°40'W.
10. chc	M	3	458311	*Okada 4851*	Argentina. Salta: Rosario de Lerma, Quebrada del Toro, Incamayo, Route 51; 2,280 m; 24°45'S, 65°44'W.
11. chc	M, D (3), R (3)	3	472820	*Okada 5608*	Argentina. Salta: Rosario de Lerma, Quebrada del Toro, Incamayo, Finca San Miguel; 2,280 m; 24°45'S, 65°44'W.
12. chc	M	3	472821	*Okada 5611*	Argentina. Salta: Rosario de Lerma, Quebrada de El Toro, 4 km E of Chorrillos; 2,150 m; 24°48'S, 65°42'W.
13. chc	M, D (3)	3	472822	*Okada 5613*	Argentina. Salta: Rosario de Lerma, Quebrada del Toro, Virrey Toledo; 1,600 m; 24°54'S, 65°39'W.
14. chc	M, R (3)	3	472823	*Okada 5616*	Argentina. Salta: Rosario de Lerma, near El Puyil, 2 km W of Campo Quijano; 1,700 m; 24°58'S, 65°45'W.
15. chc	M	4	275139	*Hjerting 56*	Argentina. Salta: Chicoana, between Chicoana and Esciope, km 16.5, near Mal Paso; 1,650 m; 25°10'S, 65°35'W.
16. chc	M	4	275140	*Hjerting 356*	Argentina. Salta: La Viña, near Moldes, km 1556; 1,100 m; 25°30'S, 65°40'W.
17. chc	M	4	275141	*Hjerting 357*	Argentina. Salta: La Viña, Ampascachi; 1,200 m; 25°30'S, 65°40'W.
18. chc	M	4	275142	*Hjerting 370*	Argentina. Salta: Guapichas, Estancia Pampa Grande, near the principal building and the represa; 1,700 m; 25°40'S, 65°30'W.
19. chc	M	4	320290	*Hawkes et al. 3629*	Argentina. Salta: Guapichas, Route 9 from Ruiz de Los Llanos to Pampa Grande at km 41.6; 1,100 m; 26°00'S, 65°27'W.
20. chc	M	4	320291	*Hawkes et al. 3633*	Argentina. Salta: Guapichas, Route 9 from Ruiz de Los Llanos to Pampa Grande at km 69.5; 1,650 m; 25°54'S, 65°30'W.

TABLE 1.   Continued

Taxcn[1]	Study[2]	Area[3]	PI[4]	Collector	Locality
21. chc	M	4	320292	*Hawkes et al. 3700*	Argentina. Salta: Chicoana, 28.8 km from Carril and 4 km above Mal Paso; 1,700 m; 25°09′S, 65°43′W.
22. chc	M	4	320293	*Hawkes et al. 3706*	Argentina. Salta: Chicoana, road from Carril to Cuesta del Obispo, pie de la Cuesta; 2,400 m; 25°09′S, 65°43′W.
23. chc	M	4	414144	*Okada 2955*	Argentina. Salta: Guapichas, Route 9, Pampa Grande; 1,750 m; 25°54′S, 65°30′W.
24. chc	M, D (2), R (3)	4	472816	*Okada 4907*	Argentina. Salta: Chicoana, Quebrada de Escoipe, Hostería El Maray; 2,500 m; 25°11′S, 65°47′W.
25. chc	M	4	472824	*Okada 5622*	Argentina. Salta: Chicoana, Quebrada de Esciope, 1 km W of Mal Paso; 1,600 m; 25°09′S, 65°38′W.
26. chc	M	4	472825	*Okada 5628*	Argentina. Salta: Chicoana, Río Malacante, 8 km W of Mal Paso; 1,800 m; 25°09′S, 65°44′W.
27. chc	M	4	472826	*Okada 5630*	Argentina. Salta: Chicoana, 2 km above Esciope, Route 59; 2,000 m; 25°11′S, 65°48′W.
28. chc	M	4	473402	*Weiseth s. n.*	Argentina. Salta: farm at Pampa Grande; 1,700 m; 25°00′S, 64°30′W.
29. chc	M	4	500042	*Okada 7490*	Argentina. Salta: Chicoana, Quebrada de Esciope, Malcante, near the bridge on Route 59; 2,000 m; 25°09′S, 65°43′W.
30. chc	M, D (3), R (3)	5	414153	*Bordas s. n.*	Paraguay. [Capital]: Lambare; [25°21′S 57°39′W].
31. chc	M	6	275137	*Hjerting 1889*	Argentina. Tucumán: Tafí, Yerba Buena, Finca Molle; 600 m; 23°46′S, 65°03′W.
32. chc	M, D (3), R (3)	6	320289	*Hawkes et al. 3432*	Argentina. Tucumán: Tafí, road from Tapia to Siambón, Raco, near km 24, Huerta La Raquefia; 1,150 m; 26°35′S, 65°45′W.
33. chc	M	7	275138	*Hjerting 56*	Argentina. Tucumán: Chicligasta, Quebrada de Las Pavas, between Puesto La Cascada and Las Cuevas; 3,000 m; 27°20′S, 65°35′W.
34. chc	M	8	265576	*Correll A707*	Argentina. Catamarca: Río Potrerillo; 29°24′S, 70°00′W.
35. chc	M	8	472832	*Okada 6888*	Argentina. Catamarca: Paclín La Viña, Route 38; 750 m; 28°01′S, 65°36′W.
36. chc	M	8	473404	*Hjerting 6377*	Argentina. Catamarca: Andagalá, Alto de Las Juntas, Route 65; 1,550 m; 27°26′S, 66°00′W.
37. chc	M, D (2), R (3)	8	473405	*Hjerting 6378*	Argentina. Catamarca: Andagalá, town of Andagalá, Route 65 near Arroyo Charquiadero; 1,400 m; 27°25′S, 66°00′W.
38. chc	M	9	472827	*González 4*	Argentina. Corrientes: Capital, Facultad de Agronomía and Veterinaria; 55 m; 27°28′S, 58°50′W.
39. chc	M	9	498325	*Okada 7309*	Argentina. Corrientes: San Cosme, Paraje El Rincón, 4 km on Route 12, near Puerto González; 50 m; 27°21′S, 58°32′W.
40. chc	M	11	458312	*Okada 4955*	Argentina. Catamarca: Pomán, Rosario de Colona, 11 km N of Pomán; 1,600 m; 28°22′S, 66°10′W.
41. chc	M, D (3), R (3)	11	458313	*Okada 4956*	Argentina. Catamarca: Pomán, Mutquín, 4 km N of Rosario de Colana; 1,700 m; 28°19′S, 66°10′W.
42. chc	M	11	458314	*Okada 4957*	Argentina. Catamarca: Pomán, Mutquín, 4 km N of Rosario de Colona; 1,700 m; 28°19′S, 66°10′W.
43. chc	M	11	472817	*Okada 4954*	Argentina. Catamarca: Pomán, Rosario de Colona, 11 km N of Pomán; 1,600 m; 28°22′S, 66°10′W.
44. chc	M	11	473406	*Hjerting 6440*	Argentina. Tucumán: Graneros, 5 km S of Rumi Punco; 500 m; 27°57′S, 65°33′W.

TABLE 1. Continued

Taxcn[1]	Study[2]	Area[3]	PI[4]	Collector	Locality
45. chc	M, D (2), R (3)	12	472819	*Okada 5341*	Argentina. Catamarca: El Alto, Guayamba, Route 64; 900 m; 28°21′S, 65°25′W.
46. chc	M, D (3), R (3)	13	498323	*Okada 5023 × 5024*	Argentina. La Rioja: San Blas de Los Sauces; 1,100 m; 28°35′S, 67°13′W.
47. chc	M	14	498320	*Okada 2838 × 2839*	Argentina. La Rioja: 2838 = Sanagasta, La Rioja, 6 km N of Huaco Abajo; 1,200 m, 29°12′S, 67°05′W. 2839 = 3 km N of Las Peñas, 1,300 m; 29°12′S, 67°05′W.
48. chc	M	15	458315	*Okada 5319*	Argentina. La Rioja: Capital, Quebrada del Sauce, Sierra de Velasco, Route 75, 10 km W of La Rioja; 700 m; 29°24′S, 66°55′W.
49. chc	M, R (3)	15	458316	*Okada 6045*	Argentina. La Rioja: Capital, Sierra de El Velasco, 8 km W on Route 38, near Las Higuerillas and El Durazno; 29°05′S, 67°05′W.
50. chc	M	15	472818	*Okada 5318*	Argentina. La Rioja: Capital, Sierra de Velasco, El Duraznillo; 1,050 m; 29°14′S, 66°52′W.
51. chc	M	15	472828	*Okada 6113*	Argentina. La Rioja: Capital, El Duraznillo, 12 km on Route 38, then 7 km N to La Rioja; 1,000 m; 29°14′S, 66°51′W.
52. chc	M	15	472829	*Okada 6116*	Argentina. La Rioja: Capital, El Duraznillo, 12 km on Route 38, then 7 km N to La Rioja; 1,100 m; 29°14′S, 66°51′W.
53. chc	M, D (3), R (3)	15	472830	*Okada 6117*	Argentina. La Rioja: Capital, El Duraznillo, 12 km on Route 38, then 7 km N to La Rioja; 1,140 m; 29°14′S, 66°51′W.
54. chc	M	15	472831	*Okada 6118*	Argentina. La Rioja: Capital, El Duraznillo, 12 km on Route 38, then 7 km N to La Rioja; 1,300 m; 29°14′S, 66°51′W.
55. chc	M	16	320285	*Hawkes et al. 3198*	Argentina. Córdoba: San Javier, 7 km from Route 148, 4.3 km E of La Paz; 650 m; 32°13′S, 65°07′W.
56. chc	M	16	320288	*Hawkes et al. 3327*	Argentina. Córdoba: Cruz del Eje, Road from Tanti to Salsacate, Río Guasta, Sierra Grande; 1,700 m; 31°19′S, 64°52′W.
57. chc	M	16	458310	*Okada 4812*	Argentina. Córdoba: Santa Rita Calamuchita, Cumbrecita, Cumbres de Achala; 1,200 m; 31°54′S, 64°46′W.
58. chc	M	16	472813	*Okada 4810*	Argentina. Córdoba: Santa Rosa Calamuchita, Villa Alpina, 38 km W of Villa General Belgrano; 1,100 m; 31°57′S, 64°47′W.
59. chc	M	17	320286	*Hawkes et al. 3297*	Argentina. Córdoba: Punilla, near de El Monte, Sierra Chica, path up Cerro Uritorco; 1,550 m; 30°51′S, 64°30′W.
60. chc	M	17	320287	*Hawkes et al. 3305*	Argentina. Córdoba: Punilla, 13 km N of Cosquín on road from La Falda to Cosquín; 850 m; 31°08′S, 64°29′W.
61. chc	M, D (3), R (3)	17	472812	*Okada 4809*	Argentina. Córdoba: Río Cuarto, El Cano, 18 km N of Alpha Corral on Route 23; 600 m; 32°37′S, 46°36′W.
62. chc	M	18	472809	*Hawkes et al. 3187*	Argentina. San Luis: General Pedernera, Cerro del Moro, S side of Estancia La Morena; 1,300 m; 33°12′S, 65°25′W.
63. chc	M	19	320282	*Hawkes et al. 3164*	Argentina. San Luis: La Capital, road from San Luis to Trapiche, above El Dique, 22 km NE of San Luis; 900 m; 33°15′S, 66°11′W.

TABLE 1.   Continued

Taxon[1]	Study[2]	Area[3]	PI[4]	Collector	Locality
64. chc	M	19	320283	*Hawkes et al. 3194*	Argentina. San Luis: Chacabuco, road from Villa del Cármen to Merlo, 1 km along a side road to the W towards Estanzuela; 900 m; 32°50′S, 65°03′W.
65. chc	M	19	320284	*Hawkes et al. 3196*	Argentina. San Luis: Chacabuco, between Larca and Merlo, 3.8 km S of San Miguel, E of Balcarce; 1,000 m; 32°34′S, 65°00′W.
66. chc	D (3)	20	n/a[6]	*Clausen et al. 435*	Argentina. Buenos Aires. [near sea level]
67. chc	M, D (3), R (3)	20	320294	*Hawkes et al. 4039*	Argentina. Buenos Aires: Castelar, Centro Nacional de Investigaciones Agropecuarias de INTA; 20 m; 34°40′S, 58°40′W.
68. mcd	M, R (3)	1	265881	*EBS[7] 1802*	Argentina. Jujuy: Quebrada de Yala; 1,800 m; 24°07′S, 65°23′W.
69. mcd	M	1	320304	*Hawkes et al. 3777*	Argentina. Jujuy: Tumbaya, Volcán, western slopes of cordillera on E side of Quebrada Grande, path from Loma de Tambo to Abra Grande de Volcán; 3,080 m; 23°55′S, 65°25′W.
70. mcd	M, D (2), R (3)	1	320305	*Hawkes et al. 3781*	Argentina. Jujuy: Tumbaya, Volcán, W facing slopes on E side of Quebrada Grande, path from Volcán to Abra Grande de Volcán; 2,960 m; 23°55′S, 65°25′W.
71. mcd	M, R (3)	1	500040	*Okada 7621*	Argentina. Jujuy: Tilcara, above Puesto Encruci- jadas on the trail to Tilcara; 23°30′S, 65°20′W.
72. mcd	M, D (2), R (3)	3	500036	*Okada 7541*	Argentina. Salta: Rosario de Lerma, Abra de Tinjas, 14 hours by mule from Castellanos; 3,350 m; 24°37′S, 65°35′W.
73. mcd	M	4	275151	*Hjerting 364*	Argentina. Salta: Iruya, between San Pedro and Rodeo Colorado; 2,800 m; 22°48′S, 64°55′W.
74. mcd	M	4	320313	*Hawkes et al. 3663*	Argentina. Salta: Guapichas, Estancia Pampa Grande, Cerro Cristal; 2,600 m; 25°50′S, 65°35′W.
75. mcd	M, D (3), R (3)	6	218224	*EBS[7] 447*	Argentina. Tucumán: Tafí, near Tucumán at Trancas; 2,500 m; 26°35′S, 65°45′W.
76. mcd	M, R (3)	8	458353	*Okada 2896*	Argentina. Catamarca: Ambato, Route 62, Las Chacritas; 1,800 m; 28°00′S, 65°55′W.
77. mcd	M, D (3), R (3)	8	558218	*Spooner & Clausen 463*	Argentina. Catamarca: Andagalá, La Banda de la Mesa de Piedra, Estancia El Chueque, S of Río Vallecito; 2,505 m; 27°19′S, 66°02′W.
78. mcd	M, R (3)	10	473178	*Okada 6092*	Argentina. Catamarca: Capital, 10 km N from Las Juntas; 27°00′S, 67°00′W.

m-1,300 m, 1,301 m-1,600 m, 1,601 m-1,799 m, 1,800 m-2,299 m, 2,300 m and above. The second subset analyzed differences within *S. chacoense* by two groups of latitudinal ranges: 1) map localities 1–6, 7–20,   and   2) map localities 1–3, 4–7, 8–11, 12–16, 17–18, 19–20.

In addition to the multivariate procedures to test for morphological intermediacy (CVA, PCO, PCA), we used the univariate "character-count proce- dure" suggested by Wilson (1992) and later applied by Thébaud and Abbott (1995). Wilson (1992)

argued, based on computer simulations, that hybridity could be distinguished from divergence by tabulating intermediate and non-intermediate character states, followed by a one-sided sign test (Zar 1984). Wilson (1992) counts a character state as intermediate even if the putative hybrid is not significantly different from one or both of the parents. For this test, we analyzed significant differences of individual characters between taxa by analysis of variance and the Tukey-Kramer test (P≤0.05) in JMP software (SAS Institute Inc. 1995).

TABLE 2.   Characters used in the morphological analysis. All measurements for the quantitative characters are in mm, except character 38 in cm. See Materials and Methods for quantitative color codes for characters 26, 27.

Leaf characters
1) Length of primary lateral leaflet.
2) Ratio: length of primary lateral leaflet/width of primary lateral leaflet at widest point.
3) Ratio: distance from apex to widest part of primary lateral leaflet/length of primary lateral leaflet.
4) Width of primary lateral leaflet from a point 5 mm below apex.
5) Length of primary lateral leaflet petiolule.
6) Length of terminal leaflet.
7) Ratio: length of terminal leaflet/width of terminal leaflet at widest point.
8) Ratio: distance from apex to widest part of terminal leaflet/length of terminal leaflet.
9) Width of terminal leaflet from a point 5 mm below apex.
10) Length of terminal leaflet petiolule.
11) Length of leaf.
12) Ratio: length of leaf/width of leaf at widest point.
13) Ratio: distance from apex to widest part of leaf/length of leaf.
14) Length of leaf petiole.
15) Adaxial pubescence: glabrous (0), puberulent (1), puberulent to canescent (2), canescent (3), pubescent (4).
16) Abaxial pubescence: glabrous (0), puberulent (1), puberulent to canescent (2), canescent (3), pubescent (4).
17) Number of leaflet pairs.
18) Number of interjected leaflets.
Stem characters
19) Width of stem wing near base of stem.
20) Stem wing morphology: wings absent (0), straight (1), wavy (2).
21) Diameter of stem near base.
22) Stem color: green (0), more green than purple (1), more purple than green (2), purple (3).
Flower characters
23) Length of pedicel.
24) Ratio: length of pedicel from base to articulation/length of pedicel.
25) Length of anther.
26) Adaxial corolla color (0–4).
27) Abaxial corolla color score minus darkest abaxial corolla color score.
28) Radius of corolla.
29) Ratio: length from center of corolla to base of corolla lobe/radius of corolla.
30) Ratio: width of corolla lobe at base of junction of corolla lobes/length from lobe at base to tip of corolla lobe.
31) Width of calyx lobe at base of junction of lobe.
32) Length of calyx lobe.

TABLE 2.   Continued

33) Length of calyx acumen.
34) Density of calyx pubescence: glabrous (0), puberulent (1), puberulent to canescent (2), canescent (3), pubescent (4).
35) Length of peduncle.
36) Number of peduncle forks per inflorescence.
37) Number of flowers per inflorescence.
Habit
38) Height of plant.

RFLP's.

SPECIES.   A total of 42 individuals of 15 accessions of *S. chacoense* and 10 individuals of four accessions of *S. microdontum* were analyzed. For each accession, one to three individuals were examined, for a total of 52 individuals (Table 1). All but one of these 19 accessions also were included in the morphological analyses. All accessions were obtained from NRSP-6 except one accession of *S. chacoense* (accession 66, Table 1) that was obtained from the Argentinian National Genebank, Balcarce.

PROBES.   A random genomic library from the wild potato *Solanum phureja* Juz. and Bukasov was used as described by Hosaka and Spooner (1992). A total of 16 clones (P43, P82, P135, P209, P215, P247, P256, P265, P274, P278, P279, P368, P374, P403, P648 and P747) was used for RFLP analysis. The clones were amplified by the Polymerase Chain Reaction and radiolabeled with ^{32}P-dCTP by the method of Feinberg and Vogelstein (1984).

DNA ISOLATION AND RESTRICTION SITE COMPARISON.   Three to five g of leaf tissue from individual plants were used for DNA extraction. All isolation and purification protocols followed those in Giannattasio and Spooner (1994) except that 6× CTAB was substituted for 2× CTAB. Five µg of each DNA sample was digested with *Dra*I and *Eco*RI restriction endonucleases according to manufacturer's instructions. Gel electrophoresis, Southern transfers, and autoradiography followed methods in Giannattasio and Spooner (1994). Hybridization and prehybridization protocols follow Havey (1991).

DATA ANALYSIS.   The operational taxonomic unit (OTU) was the individual. Polymorphic bands were converted to one (presence) and zero (absence) data. A total of 5.1% of the data was scored as missing. Only clearly visible bands were scored from only one enzyme per probe. PCA of all individuals was performed from a Jaccard's similarity matrix (J) and EIGEN using NTSYS-pc®, version 1.70 (Rohlf 1992).

PCA also was conducted on subsets of the data

used in the morphological analysis. Chi-square was used to test statistical support among *S. microdontum* and elevational groups of *S. chacoense* as discovered in this study and described in Results. The null hypothesis is that *S. microdontum* and the two elevational groups of *S. chacoense* are the same. The observed number of shared fragments within groups for each band was compared to the expected number of shared fragments, if groups were identical and individual Chi-square values per band are pooled over all bands. The data also were analyzed by visual inspection for additivity of bands.

RAPD's.

SPECIES. A total of 42 individuals of 14 accessions of *S. chacoense* and 24 individuals of eight accessions of *S. microdontum* were analyzed. For each accession three individuals were examined for a total 66 individuals (Table 1). All accessions were included in the morphological analyses, and all but seven were included in the RFLP analysis (Table 1).

PRIMERS, DNA ISOLATION, AND DATA ANALYSIS. DNA was isolated as above. A total of 33 10-mer Operon primers (Operon Technologies, Alemada, California) were selected based on clearly discernible polymorphic bands: opa4, opa5, opa12, opa14, opa15, opa18, opb14, opd4, opd20, ope18, opm2, opr7, opr8, opr9, opr12, opr13, ops3, ops7, ops9, ops11, ops17, ops19, opt1, opaa2, opaa10, opaa12, opaa14, opab4, opac5, opae5, opag2, opan15, and opao1.

RAPD amplifications were performed in a Perkin Elmer 9600 Thermal Cycler programmed for 40 cycles. The cycling protocol followed procedures listed in Spooner et al. (1996). Data analyses were the same as with the RFLP's. A total of 6.1% of the data was scored as missing.

RESULTS

Morphological Analyses. All morphological analyses showed very similar results of the PCO and PCA, and PCA will be the only results discussed. PCA of the morphological data of *S. chacoense* (not shown) separated *S. chacoense* into two widely interdigitating elevational groups, above and below 1,500 m (but not the other three elevational subsets as described in Materials and Methods). Despite much interdigitation of groups, the F-statistic for Mahalanobis Distance of the CVA was significant at P=0.04. The SDA distinguished the higher elevational group from the lower elevational group by seven morphological charac-

ters in decreasing discriminate ability: denser leaf pubescence, wider terminal leaflet apex, more purple stem color, longer calyx lobe, smaller ratio of distance from apex to widest point of primary lateral leaflet/length of primary lateral leaflet, shorter petiole, and larger ratio of distance from apex to widest point of terminal leaflet/length of terminal leaflet.

PCA of the morphological data (not shown) similarly separated *S. chacoense* only into two widely interdigitating latitudinal groups (map areas 1–6, northern populations; populations 7–20, southern populations), but not the six groups as outlined in Materials and Methods. Despite this interdigitated separation, the F-statistic for Mahalanobis Distance of the CVA was significant at P=0.0004. Of the 32 accessions in northern populations, 23 were from the high elevational group (above 1,500 m); of the 34 accessions in the southern populations, only eight accessions were from the high elevational group (above 1,500 m). The SDA separated the latitudinal groups by the following 12 morphological characters in decreasing discriminant ability: denser leaf pubescence, longer primary lateral leaflet, wider calyx lobe, more wavy stem wing, longer pedicel, longer radius of corolla, smaller ratio of width of corolla lobe at base of junction of corolla lobes/length from lobe at base to tip of corolla lobe, more purple stem color, wider primary lateral leaflet apex, longer terminal leaflet, smaller ratio of length of terminal leaflet/width of terminal leaflet at widest point, and taller plant (Table 2).

PCA of the entire morphological data set clearly separated *S. microdontum* from *S. chacoense* (Fig. 2). The first two PCA axes account for 22% and 12% of the total variation, respectively. The third PCA axis accounts for 9% variation, but does not change the overall pattern and is not presented. Axis 1 is most highly influenced by ratio of length of terminal leaflet/width of terminal leaflet at widest point, number of leaflet pairs, ratio of distance from apex to widest part of primary lateral leaflet/length of the leaflet, length of lateral leaflet, and length of primary lateral leaflet petiolule; axis 2 is most highly influenced by ratio of distance from apex to widest part of leaf/length of leaf, width of calyx lobe at base of junction of lobe, length of peduncle, length of leaf petiole, and length of leaf (Table 2). This PCA shows no evidence for the overall morphological intermediacy of upland populations of *S. chacoense* (above 1,500 m) relative to the lowland populations (below 1,500 m) and *S.*
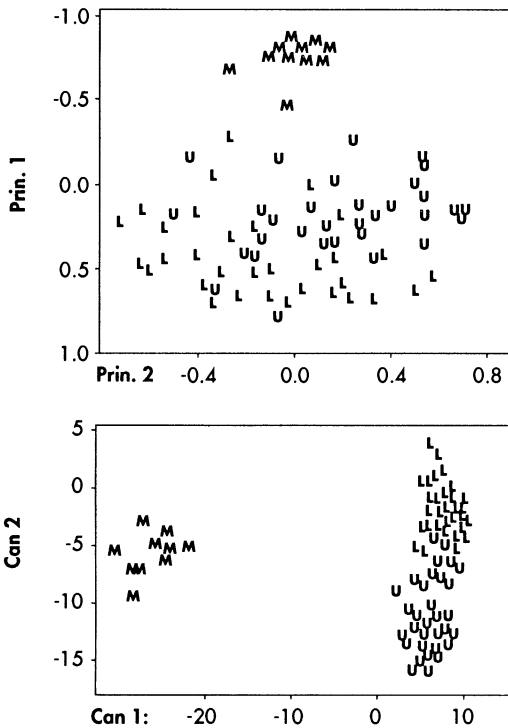
FIGS. 2, 3. Principal Components Analysis and Canonical Variates Analysis based on 38 morphological characters (Table 2) of *Solanum microdontum* (M), *S. chacoense* lowland populations (L, below 1,500 m), *Solanum chacoense* upland populations (U, above 1,500 m). 2. Principal Components axes 1,2. 3. Canonical Variates axes 1,2.
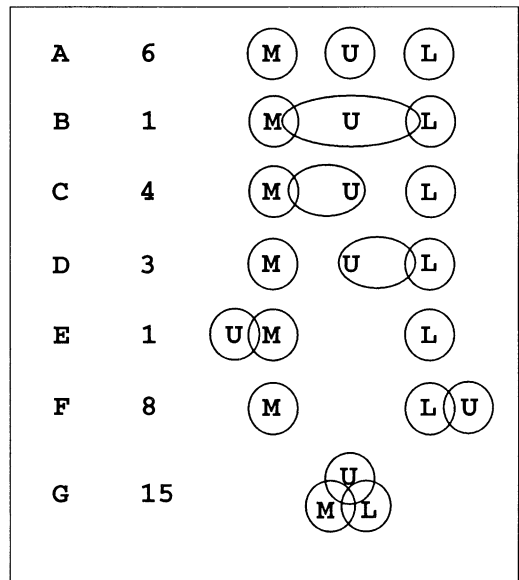

FIG. 4. Relative character state distributions of the means of 38 morphological characters among *S. microdontum* (M), *S. chacoense* lowland populations (L, below 1,500 m), *S. chacoense* upland populations (U, above 1,500 m). The letters A-G represent character classes as discussed in the text, followed by the number of characters in each class, followed by the relative population means. Intersecting and non-intersecting circles represent statistically significant differences and non-statistically significant differences between groups, respectively.

microdontum. It shows many accessions from both elevation categories both near *S. microdontum* and distant from it.

CVA (Fig. 3) distinguishes all three groups (F statistic among all groups P<0.001), with both elevational categories of *S. chacoense* equidistant to *S. microdontum* (Fig. 3), similarly providing no support for overall morphological intermediacy. Axis 1 is most highly influenced by length of calyx acumen, density of calyx pubescence, number of leaflet pairs, length of terminal leaflet, and length of calyx lobe; axis 2 is most highly influenced by characters length of leaf petiole, stem color, abaxial leaf pubescence, adaxial leaf pubescence, and length of primary lateral leaflet petiolule (Table 2).

The character count procedure (Wilson 1992) rejects the morphological intermediacy of the upland populations of *S. chacoense*. Analysis of variance shows statistically significant differences (P≤0.05) between the parents for 23 (character classes A-F; Fig. 4) of the 38 morphological

characters (Table 3). Upland *S. chacoense* is intermediate and significantly different than both putative parents in six (A) of the 23 characters (length of primary lateral leaflet petiolule, adaxial leaf pubescence, abaxial leaf pubescence, width of stem wing near base of stem, length of calyx lobe, length of calyx acumen). Upland *S. chacoense* is intermediate in one character (B; length of leaf), but not significantly different from either parent. Four characters (C; stem wing morphology, diameter of stem near base, stem color, width of calyx lobe at base of junction of lobe) show intermediacy of upland *S. chacoense* but not significantly different from *S. microdontum*. Three characters (D; length of terminal leaflet, number of leaflet pairs, density of calyx pubescence) were intermediate but not significantly different from lowland *S. chacoense*. One character (E; length of leaf petiole) was transgressive beyond *S. microdontum* but not significantly so, and eight characters (F; length of primary lateral leaflet, ratio of length of primary lateral leaflet/width of primary lateral leaflet at widest

TABLE 3. Means and standard deviations (in parentheses); difference among means (ns = not significant, sig = significant, p ≤ 0.05); and designation of intermediacy; of lowland populations of *S. chacoense* (below 1,500 m), upland populations of *S. chacoense* (above 1,500 m), and *S. microdontum*, for the 23 morphological characters that are significantly different between *S. microdontum* and lowland populations of *S. chacoense..*

Characters and character classes (see Table 2, Fig. 4)	Lowland *S. chacoense*	Upland *S. chacoense*	*S. microdontum*	Significance, lowland *S. chacoense* to upland *S. chacoense*	Significance, upland *S. chacoense* to *S. microdontum*	Intermediate, upland *S. chacoense* to lowland *S. chacoense* and *S. microdontum*
1 (F)	58.81 (2.26)	60.66 (2.33)	22.71 (3.98)	ns	sig	no
2 (F)	2.11 (0.064)	2.23 (0.066)	0.83 (0.011)	ns	sig	no
3 (F)	0.65 (0.04)	0.707 (0.04)	0.324 (0.06)	ns	sig	no
5 (A)	4.53 (0.31)	3.28 (0.32)	0.99 (0.54)	sig	sig	yes
6 (D)	62.9 (2.06)	67.22 (2.13)	130 (3.63)	ns	sig	yes
7 (F)	1.94 (0.04)	2 (0.42)	1.51 (0.072)	ns	sig	no
9 (F)	6.4 (0.29)	5.51 (0.3)	8.44 (0.52)	ns	sig	no
10 (F)	14.34 (0.82)	12.8 (0.85)	23.1 (1.45)	ns	sig	no
11 (B)	221.6 (8.58)	210.8 (8.84)	177.25 (15.08)	ns	ns	yes
12 (F)	1.608 (0.05)	1.543 (0.05)	1.893 (0.08)	ns	sig	no
14 (E)	40 (1.85)	30.32 (1.91)	37.14 (3.26)	sig	ns	no
15 (A)	0.161 (0.08)	1 (0.08)	2.73 (0.14)	sig	sig	yes
16 (A)	0.235 (0.08)	1.093 (0.09)	2.66 (0.15)	sig	sig	yes
17 (D)	4.75 (0.11)	4.68 (0.12)	0.673 (0.2)	ns	sig	yes
18 (F)	5.4 (0.64)	6.27 (0.66)	0.02 (0.00)	ns	sig	no
19 (A)	1.44 (0.12)	1.97 (0.13)	3.96 (0.22)	sig	sig	yes
20 (C)	1.5 (0.05)	1.75 (0.05)	1.97 (0.09)	sig	ns	yes
21 (C)	7.88 (0.06)	8.82 (0.26)	9.39 (0.45)	sig	ns	yes
22 (C)	1.72 (0.08)	2.24 (0.08)	2.61 (0.14)	sig	ns	yes
31 (C)	2.35 (0.05)	2.56 (0.05)	2.62 (0.09)	sig	ns	yes
32 (A)	3.01 (0.14)	3.59 (0.15)	7.8 (0.25)	sig	sig	yes
33 (A)	0.66 (0.10)	1.11 (0.1)	5.46 (0.18)	sig	sig	yes
34 (D)	1.022 (0.043)	1.06 (0.04)	2.97 (0.08)	ns	sig	yes

point, ratio of distance from apex to widest part of primary lateral leaflet/length of primary lateral leaflet, ratio of length of terminal leaflet/width of terminal leaflet at widest point, width of terminal leaflet from a point 5 mm below apex, length of terminal leaflet petiolule, ratio of length of leaf/width of leaf at widest point, number of interjected leaflets) were transgressive beyond lowland *S. chacoense* but not significantly so. Thus, only six characters (A) of upland populations of *S. chacoense* are statistically intermediate.

In our study, 23 (A-F) of the 38 characters were significantly different between *S. microdontum* and lowland populations of *S. chacoense*, but only 14 (A-D) are intermediate. Wilson's character count test requires 17 of 23 characters to be intermediate at the $P \le 0.05$ level to accept morphological intermediacy, but the 14 (A-D) intermediate characters in this study resulted in a $P=0.41$, thus failing to reject the null hypothesis of divergence.

A more stringent test of only statistically significant intermediate characters reduces the number from 14 (A-D) to six (A). Another approach would

be to test an alternative hypothesis of introgression that includes 15 (A-E) of 23 (A-F) characters showing possible introgressive influence of *S. microdontum* in upland populations of *S. chacoense*. However, this test also fails to support introgression.

RFLP Analyses. The 16 probes produced 50 scorable polymorphic bands (Miller 1994). Visual inspection of the data showed no species-specific banding patterns to any of the three groups. PCA generally clustered individuals within populations closer than individuals between populations. The first two PCA axes account for 62% and 9% of the total variation, respectively. The third PCA axis accounts for 6% variation, but does not change the overall pattern and is not presented. The RFLP PCA (Fig. 5), like the morphological PCA, clearly separates *S. microdontum* from *S. chacoense*, but shows no RFLP intermediacy in the upland populations of *S. chacoense*.

Chi-square analyses shows upland and lowland *S. chacoense* and *S. microdontum* are significantly different from each other. The lowland and upland
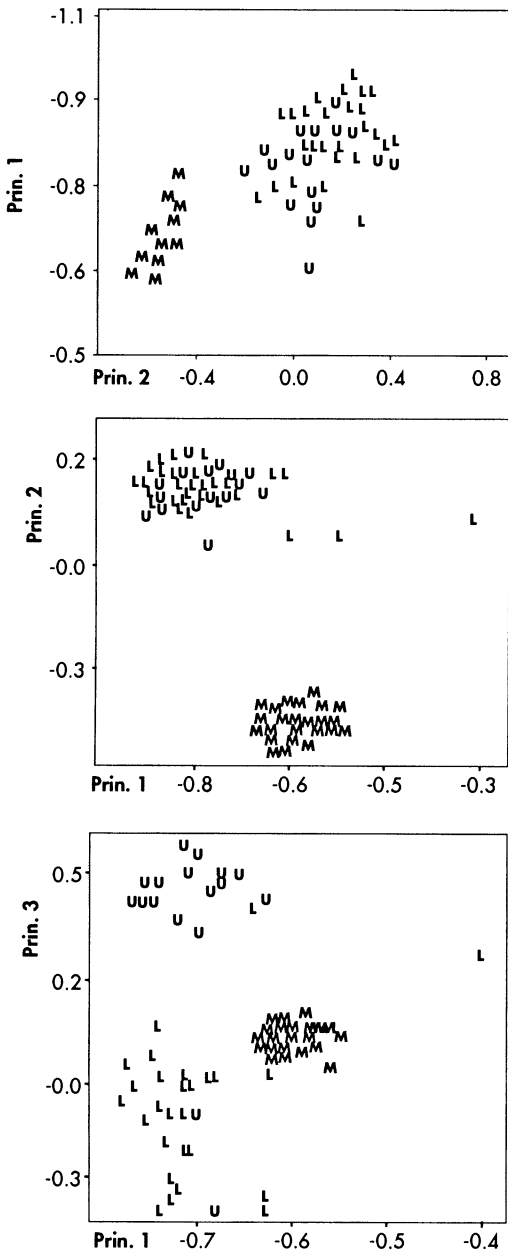
FIGS. 5–7.   Principal Components Analyses of *Solanum microdontum* (M), *S. chacoense* lowland populations (L, below 1,500 m), *S. chacoense* upland populations (U, above 1,500 m). 5. Axes 1,2, based on 50 RFLP bands. 6. Axes 1,2, based on 142 RAPD bands. 7. Axes 1,3, based on 142 RAPD bands.

populations of *S. chacoense* are differentiated at a P=0.016, while upland and lowland *S. chacoense* were differentiated from *S. microdontum* at P<0.001.

   RAPD Analyses.   The 33 primers produced 142 scorable polymorphic bands (data available from the authors). Visual inspection of the data showed species-specific banding patterns for *S. microdontum* but neither for *S. chacoense* as a species nor for the upland or lowland elevational subsets. PCA generally clusters individuals within populations closer than individuals between populations. The first two PCA axes account for 45% and 19% of the total variation, respectively. The RAPD first and second PCA axes (Fig. 6), like the morphological and RFLP PCA, clearly separate *S. microdontum* from *S. chacoense*, and provide no support for the intermediacy of upland populations of *S. chacoense*. The RAPD PCA axis three accounts for 4% variation. The RAPD first and third PCA axes (Fig. 7), with only four exceptions, separate lowland from upland *S. chacoense* while maintaining the separation of *S. microdontum*. Chi square analysis of the RAPD data parallels the RFLP data. All groups are significantly differentiated at P<0.001.

DISCUSSION

   The morphological, RFLP, and RAPD data clearly distinguish *S. microdontum* from *S. chacoense*. The morphological data distinguish lowland (below 1,500 m) from upland (above 1,500 m) populations of *S. chacoense* with difficulty only with CVA. The RFLP data fail to distinguish lowland from upland populations *S. chacoense*, and the RAPD data distinguish lowland from upland populations of *S. chacoense* with difficulty. These results do not support the origin of the upland populations of *S. chacoense* by introgressive hybridization involving *S. microdontum*.

   Hawkes (1962) was not specific regarding elevational ranges defining upland from lowland populations of *S. chacoense*, and we had to define these elevational groups here. He stated that five characters showed correlations with altitudes: broader stem wings, shorter petioles, denser pubescence, higher pedicel articulation, and longer calyx acumens. Our analysis of three elevational groups found significant morphological differences only above and below 1,500 m, providing support for his determination of *S. chacoense* being different in the uplands and lowlands. Hawkes (1962) did not include *S. microdontum* in his morphological analysis of *S. chacoense*, but designated this species as the introgressive parent because it possesses five morphological features found in the upland populations of *S. chacoense*. Our morphological analysis of upland and lowland populations of *S. chacoense* distinguished these groups by only two of the five characters mentioned by Hawkes: shorter petioles

and denser leaf pubescence, and five other morphological characters not mentioned by Hawkes (ratio of distance from apex to widest part of primary lateral leaflet/length of primary lateral leaflet, ratio of distance from apex to widest part of terminal leaflet/length of terminal leaflet, width of terminal leaflet at a point 5 mm below apex, stem color, length of calyx lobe).

Hawkes (1962) also found the five character states of upland populations of *S. chacoense* to be more common in the northern Argentinian provinces of Jujuy and Salta. Our analysis of *S. chacoense* showed significant differences in only one latitudinal group (map areas 1–6, 7–20). This corresponds to Jujuy, Salta, and adjacent northern Tucumán, likewise providing support for Hawkes's (1990) determination of latitudinal groups being different.

Brücher (1966) criticized Hawkes's *S. chacoense* introgression hypothesis. His morphological analysis of herbarium samples of *S. chacoense* showed great variation of the five "diagnostic" characters at all altitudes. He stated that *S. microdontum*, like *S. chacoense*, is extremely variable and occurs below 1,000 m, providing opportunities for introgression in lowlands, as well as uplands. He showed that plants with high hybrid indices, which Hawkes considered indicative of introgression, were found in some lowland areas, and that Hawkes generally used arguments of introgression to explain most patterns of character state variation. He criticized Hawkes's hypothesis of the rapid production of extensive introgressant populations subsequent to European colonization.

Most hypotheses of hybridization and introgression in plants have been generated from intermediate morphology and inferences from distributional data (Rieseberg and Ellstrand 1993). Some hypotheses were later tested by explicit univariate or multivariate morphological analyses, crossing data, and cytological analyses. Additive morphology is not always associated with hybridity. Rather, hybrids often show a mosaic of intermediate, parental, transgressive, or novel morphological characters (McDade 1990; Rieseberg and Ellstrand 1993). Our analysis documented all but novel character states in the upland populations of *S. chacoense*, but it showed no evidence for its introgressive origin with *S. microdontum*.

Various types of molecular markers and analyses have been used to investigate hybridization and introgression (Rieseberg and Wendel 1993). Isozymes have been used most commonly, but chloroplast DNA, nuclear ribosomal DNA, single-to low-copy nuclear DNA, and random amplified polymorphic DNA also have been used (Rieseberg and Wendel 1993). Data analyses have included visual inspection for additivity (Crawford et al. 1993), multivariate techniques such as phenograms or principal components analyses (Wolfe and Elisens 1993), intermediate values of genetic distances (dePamphilis and Wyatt 1990), discordance between chloroplast and nuclear phylogenies (Aldrich and Doebley 1992; Smith and Sytsma 1990), comparisons of discordant phenograms produced from analyses of separate regions of the nuclear genome (Debener et al. 1991), and by genetic linkage maps of natural hybrid species compared to artificially reconstructed hybrids (Rieseberg et al. 1996).

Molecular studies of hybridization rely on three criteria: 1) parental taxa must be extant and divergent at one or more loci; 2) parental taxa must not have diverged since the hybridization event, and 3) the hybrid must be of recent origin and not have diverged appreciably since its formation (Crawford 1985).

Various molecular and crossability studies suggest the very close relationship of most South American members of sect. *Petota*. For example, chloroplast DNA restriction site data show few differences within the South American (and Mexican polyploid) species (Hosaka et al. 1984; Spooner and Sytsma 1992). Also, despite some well-developed biological isolating mechanisms (Hanneman 1989), most South American species hybridize with one another and produce fertile hybrids (Ugent 1970; Hawkes 1978, 1990).

Most other molecular reinvestigations of hybridization or introgression investigated populations geographically more restricted than ours (e.g., Crawford et al. 1993; Cruzan and Arnold 1993). It is possible that population level studies of these two species would be required to document introgression, if such introgression has occurred.

In summary, our study provides no support for the hypothesis of hybridization or introgression of *S. chacoense* and *S. microdontum*. Studies of widespread natural introgression may be difficult to support in sect. *Petota* because of the similarity of many of its component taxa. Also, morphological and molecular support of hybridization can have various alternative explanations including convergence, retention of ancestral characters, and hybridization with species not here examined. Rieseberg and Wendel (1993) showed difficulties in detecting introgression: 1) hybridization is more likely between closely related species; 2) closely related species likely have fewer species-specific morpho-

logical and molecular markers, and 3) the likelihood of detecting hybridization decreases over time because the hybrid becomes "diluted" by possible further introgression with other species, mutation, and extinction of parents. All of these complicating factors could very well occur in *Solanum* sect. *Petota*.

LITERATURE CITED

ABDALLA, M. M. F. and J. G. HERMSEN. 1972. Unilateral incompatibility: hypotheses debate and its implications for plant breeding. Euphytica 21: 32–47.

ALDRICH, P. R. and J. DOEBLEY. 1992. Restriction fragment variation in the nuclear and chloroplast genomes of cultivated and wild *Sorghum bicolor*. Theoretical and Applied Genetics 85: 293–302.

BAMBERG, J. B., M. W. MARTIN, J. J. SCHARTNER, and D. M. SPOONER. 1996. *Inventory of tuber-bearing Solanum species*. Sturgeon Bay, Wisconsin: Potato Introduction Station, NSPR-6.

BRUCHER, H. 1953. Über das natürliche Vorkommen von Hybriden zwischen *Solanum simplicifolium* und *Solanum subtilis* im Aconquija Gebirge. Zeitschrift für induktive Abstammungsund Vererbungslehre 85: 12–19.

———. 1966. Gen-introgression bei *Solanum chacoense?* Biologisches Zentralblatt 85: 627–634.

CHILD, A. 1990. A synopsis of *Solanum* subgenus *Potatoe* (G. Don) (D'Arcy) (*Tuberarium* (Dun.) Bitter (s.l.)). Feddes Repertorium Novarum Regni Vegetabilis 95: 238–298.

CORRELL, D. S. 1962. *The potato and its wild relatives*. Renner, Texas: Texas Research Foundation.

CRAWFORD, D. J. 1985. Electrophoretic data and plant speciation. Systematic Botany 10: 405–416.

———, S. BRAUNER, M. B. COSNER, and T. F. STUESSY. 1993. Use of RAPD markers to document the origin of the intergeneric hybrid × *Margyracaena skottsbergii* (Rosaceae) on the Juan Fernandez Islands. American Journal of Botany 80: 89–92.

CRUZAN, M. B. and M. L. ARNOLD. 1993. Ecological and genetic associations in an *Iris* hybrid zone. Evolution. 47: 1432–1445.

DEBENER, T., F. SALAMINI, and C. GEBHARDT. 1991. The use of RFLPs (restriction fragment length polymorphisms) detects germplasm introgressions from wild species into potato (*Solanum tuberosum* ssp. *tuberosum*) breeding lines. Plant Breeding 106: 173–181.

DEPAMPHILIS, C. W. and R. WYATT. 1990. Electorphoretic confirmation of interspecific hybridization is *Aesculus* (Hippocastanceae) and the genetic structure of a broad hybrid zone. Evolution 44: 1295–1317.

FEINBERG, A. P. and B. VOLGELSTEIN. 1984. Addendum. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 137: 266–267.

FRITZ, N. K. and R. E. HANNEMAN, JR. 1989. Interspecific incompatibility due to stylar barriers in tuber-bearing and closely related non-tuber-bearing Solanums. Sexual Plant Reproduction 2: 184–192.

GIANNATTASIO, R. B. and D. M. SPOONER. 1994. A reexamination of species boundaries between *Solanum megistacrolobum* and *S. toralapanum* (*Solanum* sect. *Petota*, series *Megistacroloba*): morphological data. Systematic Botany 19: 89–105.

HANNEMAN, R. E., JR. 1989. The potato germplasm resource. American Potato Journal 66: 655–667.

———. 1994. Assignment of endosperm balance numbers to the tuber-bearing Solanums and their close non-tuber-bearing relatives. Euphytica 74: 19–25.

HAVEY, M. J. 1991. Phylogenetic relationships among cultivated *Allium* species from restriction enzyme analysis of the chloroplast genome. Theoretical and Applied Genetics 81: 752–757.

HAWKES, J. G. 1962. Introgression in certain wild potato species. Euphytica 11: 26–35.

———. 1978. Biosystematics of the potato. Pp. 15–69 in *The potato crop: the scientific basis for improvement*, ed. P. M. Harris. London: Chapman and Hall.

———. 1990. *The potato: evolution, biodiversity and genetic resources*. Washington, D.C.: Smithsonian Institution Press.

———. 1994. Origins of cultivated potatoes and species relationships. Pp. 3–42 in *Potato genetics*, eds. J. E. Bradshaw and G. R. MacKay. Wallingford, England: CAB International.

——— and J. P. HJERTING. 1969. *The potatoes of Argentina, Brazil, Paraguay and Uruguay: a biosystematic study*. Oxford: Oxford Univ. Press.

——— and ———. 1989. *The potatoes of Bolivia: their breeding value and evolutionary relationships*. Oxford: Oxford Univ. Press.

HOSAKA, K. and D. M. SPOONER. 1992. RFLP analysis of the wild potato species, *Solanum acaule* Bitter (*Solanum* sect. *Petota*). Theoretical and Applied Genetics 84: 851–858.

———, Y. OGIHARA, M. MATSUBAYASHI, and K. TSUNEWAKI. 1984. Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Japanese Journal of Genetics 59: 349–369.

MCDADE, L. 1990. Hybrids and phylogenetic systematics l. Patterns of character expression in hybrids and their

implications for cladistic analysis. Evolution 44: 1685–1700.

MILLER, J. T. 1994. A reexamination of hypotheses of hybridization concerning *Solanum chacoense* Bitter (*Solanum* sect. *Petota*). M.S. Thesis, University of Wisconsin, Madison.

RIESEBERG, L. H. 1995. The role of hybridization in evolution: old wine in new skins. American Journal of Botany 82: 944–953.

——— and N. C. ELLSTRAND. 1993. What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Sciences 12: 213–241.

——— and J. F. WENDEL. 1993. Introgression and its consequence in plants. Pp. 70–109 in *Hybrid zones and the evolutionary process*, ed. R. G. Harrison. New York: Oxford Univ. Press.

———, B. SINERVO, C. R. LINDER, M. C. UNGERER, and D. M. ARIAS. 1996. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. Science 272: 741–745.

ROHLF, F. J. 1992. *NTSYS-pc, numerical taxonomy and multivariate analysis system*. New York: Exeter Publishing, Ltd.

ROYAL HORTICULTURAL SOCIETY. 1986. R.H.S. Colour chart. [ed. 1,2] London: Royal Horticultural Society.

SAS INSTITUTE INC. 1988. *SAS® user's guide: statistics, version 5*. Cary, North Carolina: SAS Institute Inc.

———. 1995. *SAS® user's guide for JMP statistical discovery software, version 3.1*. Cary, North Carolina: SAS Institute Inc.

SMITH, R. L. and K. J. SYTSMA. 1990. Evolution of *Populus nigra* (sect. *Aigeiros*): Introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). American Journal of Botany 77: 1176–1187.

SPOONER, D. M. and K. J. SYTSMA. 1992. Reexamination of series relationships of Mexican and Central American wild potatoes (*Solanum* sect. *Petota*): evidence from chloroplast DNA restriction site variation. Systematic Botany 17: 432–448.

——— and R. G. VAN DEN BERG. 1992a. An analysis of recent taxonomic concepts in wild potatoes (*Solanum* sect. *Petota*). Genetic Resources and Crop Evolution 39: 23–37.

——— and ———. 1992b. Species limits and hypotheses of hybridization of *Solanum berthaultii* Hawkes and *S. tarijense* Hawkes: morphological data. Taxon 41: 685–700.

———, G. J. ANDERSON, and R. K. JANSEN. 1993. Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). American Journal of Botany 80: 676–688.

———, J. TIVANG, J. NIENHUIS, J. T. MILLER, D. S. DOUCHES, and A. CONTRERAS M. 1996. Comparison of four molecular markers in measuring relationships among the wild potato relatives *Solanum* section *Etuberosum* (subgenus *Potatoe*). Theoretical and Applied Genetics 92: 532–540.

THÉBAUD, C. and R. J. ABBOTT. 1995. Characterization of invasive *Conyza* species (Asteraceae) in Europe: quantitative trait and isozyme analysis. American Journal of Botany 82: 360–368.

TUCKER, A. O., M. J. MACIARELLO, and S. S. TUCKER. 1991. A survey of color charts for biological descriptions. Taxon 40: 201–214.

UGENT, D. 1970. The potato. Science 170: 1161–1166.

VAN DEN BERG, R. G. and D. M. SPOONER. 1992. A reexamination of infraspecific taxa of a wild potato, *Solanum microdontum* Bitter (*Solanum* sect. *Petota*: Solanaceae). Plant Systematics and Evolution 182: 239–252.

WILSON, P. 1992. On inferring hybridity from morphological intermediacy. Taxon 41: 11–23.

WOLFE, A. D. and W. J. ELISENS. 1993. Diploid hybrid speciation in *Penstemon* (Scrophulariaceae) revisited. American Journal of Botany 80: 1082–1094.

ZAR, J. H. 1984. *Biostatistical analysis*. Englewood Cliffs, New Jersey: Prentice Hall.