Chilean Tetraploid Cultivated Potato, *Solanum tuberosum*, is Distinct from the Andean Populations: Microsatellite Data

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ABSTRACT

This study tests the genetic difference between landrace populations of *Solanum tuberosum* L. subsp. *tuberosum* and subsp. *andigenum* (Juz. & Bukasov) Hawkes using nuclear DNA microsatellites. Microsatellite loci were amplified in subsp. *andigenum* (35 accessions), subsp. *tuberosum* (35 accessions), and other cultivated and wild species (22 accessions). A total of 208 alleles were scored from 18 microsatellite loci spread throughout all 12 chromosomes of potato. Using an infinite allele model and a least squares method of analysis, microsatellite loci separated subsp. *tuberosum* from subsp. *andigenum*, and cultivated and wild species. These results support the genetic difference of these two populations and their recognition at some classification level.

The potato and its wild relatives (*S.* sect. *Petota* Dumort.) harbor a wealth of both wild and cultivated tuber-bearing species distributed from the southwestern USA to central Argentina and adjacent Chile (Hawkes, 1990). The taxonomic treatment of section *Petota* varies greatly among authors (Spooner and van den Berg, 1992). Hawkes (1990) recognized 217 wild and seven cultivated potato species with morphological data. Spooner and Hijmans (2001) list 199 wild species. The cultivated species range in ploidy level from diploid (2n = 2x = 24) to pentaploid (2n = 5x = 60).

The Andean and Chilean tetraploid cultivated potatoes have been treated as two separate species, *S. andigenum* Juz. and Bukasov and *S. tuberosum* L.; as subspecies of *S. tuberosum*; and as two cultivar groups within *S. tuberosum* (Huamán and Spooner, 2002). Cultivar groups are taxonomic categories used by the International Code of Nomenclature of Cultivated Plants (ICNCP) to associate cultivated plants with traits that are of use to agriculturists, but imply no phylogenetic differences between the groups. Species and subspecies, in contrast, are treated by the International Code of Botanical Nomenclature (ICBN) and generally assume phylogenetic differences (Spooner et al., 2002). Subspecies *tuberosum* is indigenous to Chiloé Island, the Chonos Archipelago to the south and adjacent areas in south-central mainland Chile. Subspecies *andigenum* is indigenous to Andean South America and ranges from Venezuela to northern Chile and Argentina (Hawkes, 1990).

Swaminathan and Magoon (1961) hypothesized that subsp. *andigenum* arose through autoploidy of the cultivated diploid species *S. stenotomum* Juz. and Bukasov. Cribb and Hawkes (1986) advocated that amphiploid subsp. *andigenum* originated from a cross between diploid cultivated *S. stenotomum* and the wild *S. sparsipilum* (Bitter) Juz. and Bakasov Matsubayashi (1991) suggested that subsp. *andigenum* originated from two diploid cultivated species *S. phureja* Juz. and Bukasov and *S. stenotomum*.

The origins of Chilean populations of subsp. *tuberosum* are speculative. Juzepczuk and Bukasov (1929) proposed that subsp. *tuberosum* originated from indigenous tetraploid cultivated species *S. fonckii* Phil. ex Reich. (nomen nudeum, no locallity other than Chile designated), *S. leptostigma* Juz. ex Bukasov (type from tubers collected in Chiloé Island), and *S. molinae* Juz. (type from tubers collected on Chiloé Island). Hawkes (1990) treated these three taxa as subsp. *tuberosum*. Juzepczuk and Bukasov (1929) suggested that *S. palustre* Schltdl. (then treated as *S. brevidens* Phil.) may be a progenitor. Hawkes (1990) proposed that subsp. *andigenum* evolved into subsp. *tuberosum* after transport to Chile, parallel with the evolution of subsp. *tuberosum* from subsp. *andigenum* in Europe. On the basis of starch grains, Ugent et al. (1987) proposed the wild species *S. maglia* Schltdl. as a progenitor of subsp. *tuberosum*. Grun (1990) hypothesized that subsp. *tuberosum* evolved from a cross between subsp. *andigenum* and an unidentified wild species.

The genetic relationships and extent of genetic differentiation between *S. tuberosum* subsp. *tuberosum* and subsp. *andigenum* is unresolved. On the basis of cytoplasmic sterility factors, geographical isolation, and ecological differences, Grun (1990) suggested that subsp. *tuberosum* was distinct from subsp. *andigenum*. Hawkes (1990) distinguished the two subspecies by subsp. *tuberosum* having fewer stems with foliage aligned at a broad angle to the stem and having less-dissected leaves with wider leaflets and thicker pedicels. Chloroplast DNA (cpDNA) restriction site data documented five chloroplast genotypes (A, C, S, T, and W types) in *S. tuberosum*, which included subsp. *tuberosum* and *andigenum*. Subspecies *andigenum* has all five types and native Chilean subsp. *tuberosum* has three types: A, T, and W (Hosaka and Hanneman, 1988). The most frequently observed type in Chilean subsp. *tuberosum* is T, which is characterized by a 241-base-pair deletion (Kawagoe and Kikuta, 1991).

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Abbreviations: bp, base pairs; cpDNA, chloroplast DNA; IAM, infinite allele model; PAUP, Phylogenetic Analysis Using Parsimony; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SMM, stepwise mutation model; Tm, melting temperature.

Table 1. Accessions of *Solanum* sect. *Petota* examined. Vouchers are deposited at the herbarium of the U.S. Potato Introduction Station in Sturgeon Bay, WI.

Accession number†	Species‡	Map area§	PI¶
	Outgroups		
1	bst	1	320265
2	pnt	2	275234
3	blb	3	275187
4	abz	15	498206
5	pur	16	473501
6	mcq	18	283114
	Ingroups		
7	ver	4	558482
8	cur#	12	225651
9	phu#	12	320385
10	stn#	20	205527
11	med	21	458402
12	ajh#	28	255490
13	opl	33	473182
14	mag	37	558316
15	buk	24	414155
16	mlt	25	210055
17	can	26	442696
18	spl	23	473385
19	acs	27	230495
20	brc	29	498111
21	vid	34	472991
22	spg	36	458337
	Ingroup (*S. tuberosum* subsp. *andigena* and subsp. *tuberosum*)		
23	adg	5	243439
24	adg	6	243438
25	adg	7	243436
26	adg	8	243364
27	adg	9	243432
28	adg	10	243413
29	adg	11	243420
30	adg	12	243441
31	adg	13	243408
32	adg	14	237208
33	adg	17	292097
34	adg	19	246497
35	adg	19	246497
36	adg	20	214426
37	adg	22	214436
38	adg	26	246514
39	adg	27	230498
40	adg	28	255505
41	adg	28	281000
42	adg	29	258927
43	adg	29	258927
44	adg	29	543020
45	adg	30	498077
46	adg	30	527887
47	adg	31	245929
48	adg	32	546021
49	adg	34	473197
50	adg	34	473249
51	adg	34	473267
52	adg	35	473258
53	adg	35	473260
54	adg	35	473284
55	adg††	28	RU97-028
56	adg††	28	RU97-031
57	adg††	28	RU97-078
58	adg††	28	RU97-115
59	adg††	28	RU97-117
60	tbr	38	245319
61	tbr	39	245317
62	tbr	40	209770
63	tbr	40	209771
64	tbr	40	245816
65	tbr	40	245836
66	tbr	40	245845
67	tbr	40	595449
68	tbr	40	595450
69	tbr	40	595451
70	tbr	40	595452
71	tbr	40	595453
72	tbr	40	595455
73	tbr	40	595456
74	tbr	40	595457
75	tbr	40	595458
76	tbr	40	595459
77	tbr	40	595460
78	tbr	40	595461
79	tbr	40	595490
80	tbr	40	595491
81	tbr	40	595492
82	tbr	40	595493
83	tbr	40	245793
84	tbr	40	245795
85	tbr	40	245815
86	tbr	40	245835
87	tbr	40	245839
88	tbr	40	CF98-003
89	tbr	40	CF98-005
90	tbr	40	CF98-009
91	tbr	40	CF98-012
92	tbr	41	587108
93	tbr	41	587109
94	tbr	41	587110

(Continued next column.)

Table 1. Continued.

† The accession number corresponds to Fig. 2.

‡ Taxon abbreviations follow Spooner and Hijmans (2001) and correspond to Fig. 2. The codes, followed by species and somatic chromosome number (in parentheses) are: acs = *Solanum acroscopicum* Ochoa (24); ajh = *S.* × *ajanhuiri* Juz. and Bukasov (24); abz = *S. albornozii* Correll (24); bst = *S. brachistotrichium* (Bitter) Rydb. (24); brc = *S. brevicaule* Bitter (24); buk = *S. bukasovii* Juz. (24); blb = *S. bulbocastanum* Dunal (24); can = *S. canasense* Hawkes (24); cur = *S.* × *curtilobum* Juz. and Bukasov (48); vid = *S. gourlayi* subsp. *vidaurrei* (Cárd.) Hawkes and Hjert. (24); mag = *S. maglia* Schltdl. (36); med = *S. medians* Bitter (24); mcq = *S. mochiquense* Ochoa (24); mlt = *S. multidissectum* Hawkes (24); opl = *S. oplocense* Hawkes (24); phu = *S. phureja* Juz. and Bukasov (24); pnt = *S. pinnatisectum* Dunal (24); pur = *S. piurae* Bitter (24); spl = *S. sparsipilum* (Bitter) Juz. and Bukasov (24); spg = *S. spegazzinii* Bitter (24); stn = *S. stenotomum* Juz. and Bukasov (24); adg = *S. tuberosum* L. subsp. *andigenum* Hawkes (48); tbr = *S. tuberosum* subsp. *tuberosum* (48); ver = *S. verrucosum* Schltdl. (24).

§ The map area corresponds to Fig. 1 and 2.

¶ The six-letter numerical codes are USDA Plant Introduction Numbers from Bamberg et al. (1996); RU and CF are collections of Raker and collaborators (see text); they have herbarium vouchers at the Potato Introduction Station Herbarium, but not germplasm collections.

Cultivated species other than *S. tuberosum* subsp. *tuberosum* or subsp. *andigenum*.

†† Cultivated species collected as tubers in Bolivia by Raker and Ugarte without known chromosome numbers that likely are *S. tuberosum* subsp. *andigenum*, but possibly could be *S. stenotumum* (see text).

Microsatellite or simple sequence repeats present a relatively new and promising technique to examine the genetic difference of subsp. *tuberosum* and subsp. *andigenum*. Microsatellites are tandemly repeated short sequences of 1 to 6 base pairs (bp) in length, spread throughout the genome, highly polymorphic, and have been used to investigate relationships among closely related taxa (Goldstein and Pollock, 1997). This study is designed to test differences between the two subspecies of *S. tuberosum* using the mapped potato microsatellite loci of Milbourne et al. (1998).

MATERIALS AND METHODS

Plant Materials

Thirty-seven accessions of landrace populations of subsp. *andigenum* and 35 of subsp. *tuberosum*, which represent a wide geographical range, were used in this study (Table 1; Fig.

Fig. 1. Map showing the 41 generalized areas of the accessions of *Solanum tuberosum* subsp. *andigenum* and subsp. *tuberosum* ingroups and outgroups used in this study. Numbers are cited as generalized map areas in Table 1 and in Fig. 2.

1). Most accessions came from the USDA Potato Germplasm Bank (Bamberg et al., 1996). Raker and M.L. Ugarte collected tubers of five additional landraces near Lake Titcaca in Bolivia (Table 1; Accessions 55–59) in March 1997. Accessions 246497 and 258927 are repeated in the study, but with different seed sources. Species, accession number, origin, and somatic chromosome number (Ochoa, 1958) are listed in Table 1 and Fig. 2. Raker and J. Fernandez collected leaf samples preserved in silica gel of four additional landraces of *S. tuberosum* from Chiloé Island, Chile, in January 1998 (Table 1; Accessions 88–91).

Morphological data (Grun, 1990; van den Berg et al., 1998) and randomly amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) data (Miller and Spooner, 1999) support the *S. brevicaule* Bitter complex and other cultivated species as close relatives to both subspecies of *S. tuberosum*. These and other members of Clade 4 of Spooner and Castillo (1997) were chosen as ingroups (Table 1). Ingroups include monophlyetic groups while outgroups are comprised of distantly related relatives chosen from Clades 1, 2, and 3 of Spooner and Castillo (1997).

Seeds from the U.S. Potato Genebank were planted in a

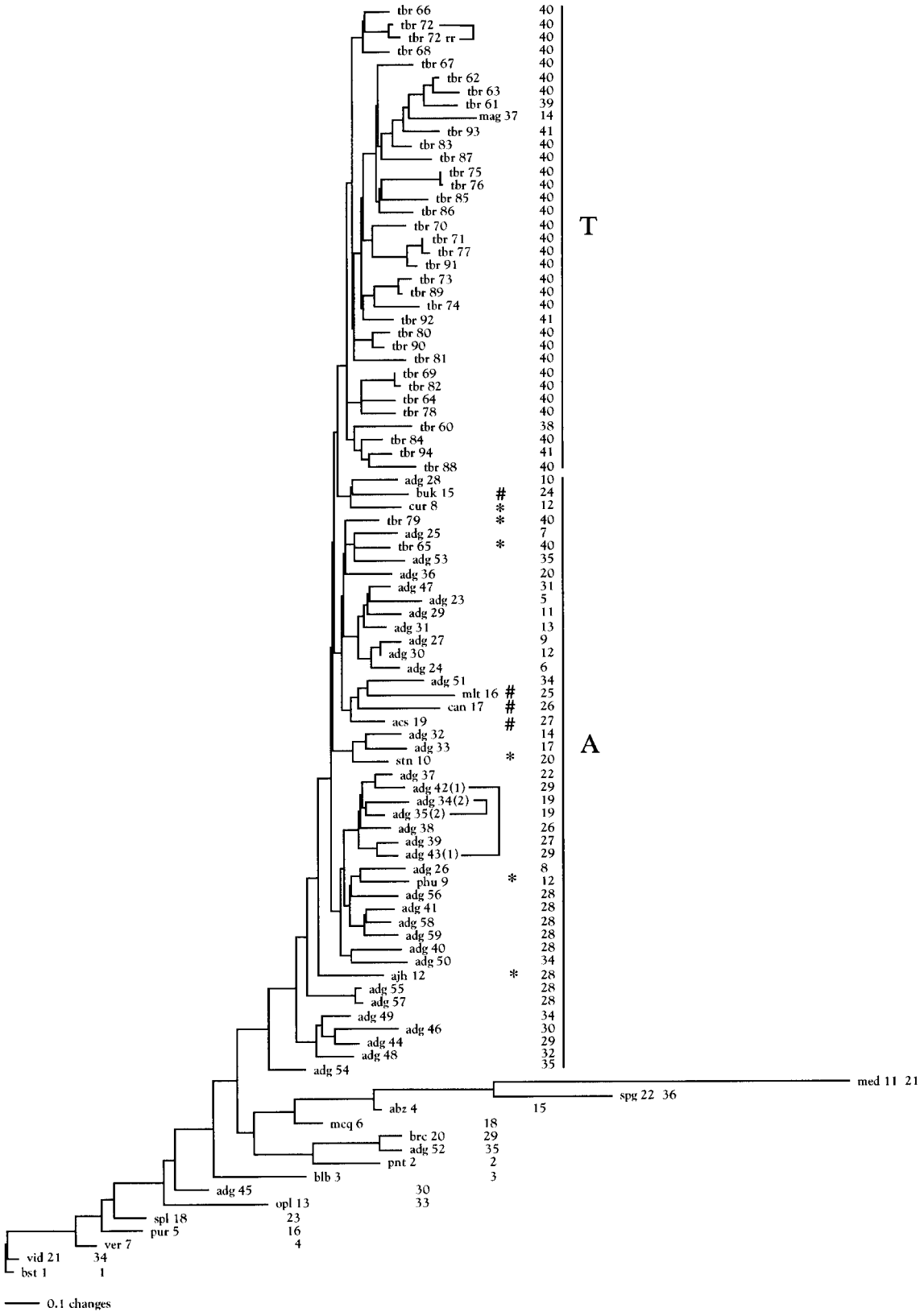

Fig. 2. Neighbor-joining tree generated from microsatellite data analyzed with Nei's (1972) similarity coefficient. The labels (left to right) are the three-letter taxon code, accession number, and generalized geographic area corresponding to Table 1 and Fig. 1. The vertical lines T and A refer to clusters of subsp. *tuberosum* and subsp. *andigenum*, respectively. Asterisks (*) and number signs (#) highlight non-subsp. *andigenum* cultivated and wild species, respectively, that fall within the subsp. *andigenum* cluster. The three sets of brackets to the right highlight accessions tbr 72 and tbr 72 rr of replicated runs of the same DNA sample, and accessions adg 42(1) and 43(1), and 34(2) and 35(2), of pairs of the same accessions with DNA extracted from a separate individual grown from a separate seed of the same accession.

greenhouse in Madison, WI, in May 1997. Tubers collected in Bolivia were planted in quarantine facilities in Glenn Dale, MD, in May 1999. Plants from both seeds and tubers were grown in pots in potting soil under ambient light. Young leaves were harvested after three months of growth. Leaves from plants grown in Maryland were sent on ice to Madison, WI, for DNA extraction. Deoxyribonucleic acid was extracted using the minipreparation methods outlined in Ballard et al. (1998). DNA concentrations were estimated by gel electrophoresis on 1% agarose gels with a low mass DNA ladder (Gibco BRL Low DNA Mass Ladder, Life Technologies, Rockford, MD)[1].

Amplification and Fragment Analysis

Primers of 23 mapped microsatellite loci from Milbourne et al. (1998) (stm0007, stm0010, stm0013, stm0019, stm0025, stm0037, stm0038, stm0051, stm0052, stm1003, stm1006, stm1008, stm1017, stm1020, stm1029, stm1031, stm1049, stm1055, stm1069, stm1100, stm1104, stm2020, and stm3016) were labeled with fluorescent dyes (FAM, HEX, TETRA from Applied Biosystems, Foster City, CA) and used to amplify DNA from one seedling per accession (Table 1). Reaction conditions were optimized as described in Provan et al. (1996). Conditions for a 25-μL reaction were as follows: 1XPCR Buffer II (Perkin Elmer, Norwalk, CT), 1.5 m*M* $MgCl_2$, 0.2 m*M* deoxyribonucleoside triphosphates, 0.4 μ*M* of each primer pair (labeled forward and unlabeled reverse), 1 unit of AmpliTaq Gold (Perkin Elmer), and 10 to 20 ng of DNA. All reactions were amplified in a Perkin Elmer 9600 thermocycler set at the following times and temperatures: 1 cycle of 10 min at 94°C, 2 min at melting temperature (Tm) (Milbourne et al., 1998), 5 min at 72°C, followed by 29 cycles of 1 min at 94°C, 45 s at Tm, 5 min at 72°C, and ending with 45 min hold at 72°C. The Tm for each primer followed Milbourne et al. (1998).

Usually, three microsatellite products from one individual were pooled by adding 3 μL of the FAM, 6 μL of TETRA, and 12 to 15 μL of HEX, depending on the strength of the product. An aliquot of 1.5 μL of each pooled sample was mixed with 1.8 μL of sample loading buffer [80% formamide, 10 mg mL^{-1} blue dextran, 5 m*M* Disodium(ethylene dinitrilo)-tetraacetic acid dihydrate pH 8.0] and 0.45 μL molecular weight standard (TAMRA 5000 standard from PE Biosystems, Wellesley, MA). Samples were heated for 3 min at 95°C, then chilled on ice. Approximately 1 μL of the chilled sample was loaded on a 5% LongRanger (FMC Bioproducts, Rockland, ME) polyacrylamide/6 *M* urea gel in a PE Biosystems 377XL DNA sequencing apparatus. Samples were run at 3000 V at 2400 scans hr^{-1} in 36-cm well-to-read plates. Data were collected using the DNA Sequencer Data Collection v. 2.0 (PE Biosystems) and analyzed with GeneScan v. 2.1 (PE Biosystems).

Fluorescent peaks were labeled as fragment sizes by using Genotyper v. 2.1 (PE Biosystems). All peaks were manually edited using Genotyper's manual click option. Peaks were scored by allele size and as presence or absence. One replicate of identical DNA (subsp. *tuberosum*, PI 595455) was included as a standard, as well as two accessions with DNA extracted from separate seedlings of the same accession.

[1] Names are necessary to report data. However, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

Data Analysis

The allelic bp scores were entered into Microsat version 1.5d (Minch et al., 1997) to generate a distance matrix using $\partial\mu^2$, the stepwise mutation model (SMM) of Goldstein et al. (1995). This model assumes that new mutations occur by stepping up or down in size within a short region of DNA, and thus could lead to a previously existing allele. We also generated distance matrices by an infinite allele model [IAM, Nei (1972) in Phylogenetic Analysis Using Parsimony (PAUP)] that assumes each mutation forms a new allele of infinite size.

Both distance matrices were entered into PAUP version 4.0d65 (Swofford, 1998), where branching trees were built using the tree building methods of unweighted pair group method with arithmetic means (UPGMA; Sokal and Sneath, 1963), neighbor-joining (Saitou and Nei, 1987), and unweighted least-squares methods (Fitch and Margoliash, 1967). All trees were built using a random input order. The unweighted least-squares tree was generated using a heuristic stepwise-addition search for 10 replicates. The distance matrices were also entered into KITSCH, a program within PHYLIP version 3.573c (Felsentein, 1995), to obtain a Fitch-Margoliash method with contemporaneous taxa (power = 2.0; 10 random replicates). Significant differences in allele distributions were determined in JMP statistical software (SAS Institute, 1995) by the Tukey-Kramer honestly significant difference (HSD) test.

RESULTS

Amplification and Products

Eighteen of the 23 primers examined were scoreable. Primer stm1020 yielded peaks that were indistinguishable from apparent PCR artifacts or 'stutter' (Schlötterer and Tautz, 1992) Primers stm0010, stm0025, and stm1100 yielded low-signal peaks that could not be identified reliably. Primer stm0013 failed to amplify well.

Of the 18 loci scored, a total of 208 alleles were detected for all 94 individuals. Both subspecies of *S. tuberosum* accounted for 46.5% of the total missing data and remaining accessions for 53.5%. Each primer produced one to four peaks per individual scored as numbers of bp for the tetraploid individuals. The diploid individuals had three identifiable peaks, with the source of the third peak not known. The number of heterozygous individuals (more than two peaks scored for one primer) across all loci ranged from 20 individuals in stm1029 to 77 individuals in stm2020, with a mean of 46.8 per locus. The size ranged from 11 bp in stm1008 to 122 bp in stm0019. The number of alleles per locus ranged from 6 in stm1069 to 19 in stm0019, with a mean of 11.6 alleles per locus.

Allelic distributions varied by ploidy, taxonomy, and taxonomic distance (ingroup vs. outgroup) (Table 2). The two subspecies of *S. tuberosum* showed no difference in mean number of null alleles per accession or mean number of heterozygous alleles per accession. However, mean number of alleles per accession were significantly ($P = 0.05$) different between the two subspecies. All other comparisons were highly significant ($P = 0.05$), with the greatest difference occurring in the number of null alleles present in the ingroup and the

Table 2. Comparison of allelic variability by ploidy, taxonomy, and phylogenetic distance (ingroup vs. outgroup).

Comparison type	Mean no. null alleles/accession	Mean no. alleles/accession	Mean no. heterozygous alleles/accession
	Subspecies of *S. tuberosum*		
subspecies *andigenum* (n = 37)	0.034	1.57*	0.51
subspecies *tuberosum* (n = 35)	0.031	1.70*	0.57
	Ploidy		
Diploid (n = 19)	0.13*	1.30*	0.34*
Tetraploid (n = 73)	0.04*	1.63*	0.54*
	***S. tuberosum*/all other species**		
S. tuberosum (n = 71)	0.04*	1.63*	0.54*
All other species (n = 22)	0.12*	1.33*	0.37*
	Ingroup/outgroup		
Ingroup (n = 88)	0.04*	1.59*	0.51*
Outgroup (n = 6)	0.25*	1.30*	0.29*

* Significantly different comparisons at $P = 0.05$.

outgroup comprised of mostly tetraploids and diploids, respectively.

Dinucleotide repeats (Milbourne et al., 1998) consistently showed two-bp differences in allele sizes, while the trinucleotide repeats varied. Primers stm1069 and stm1049 amplified fragments with two-bp differences instead of the expected three (Milbourne et al., 1998), hence we treated it as a dinucleotide repeat for the $\partial\mu^2$ analysis. Primers stm1104 and stm1017 showed a one-bp difference and we treated them as single nucleotide repeats in the $\partial\mu^2$ distance calculation. Primer stm1029, which amplifies a single nucleotide repeat region, showed alleles of two-bp differences; thus we considered it a dinucleotide repeat.

Comparison of IAM and SMM Trees

The IAM model consistently generated trees that placed the replicate sample and the two individuals from duplicate accessions near each other (Fig. 2). Conversely, all trees generated from SMM separated the replicate samples and duplicate accessions, intermixing subspecies of *S. tuberosum*, cultivated species, and *S. brevicaule* taxa.

The IAM or neighbor-joining tree (Fig. 2) separated 33 of the 35 accessions of subsp. *tuberosum*, intermixed with *S. maglia*. It placed two accessions of subsp. *tuberosum* with subsp. *andigenum* that also contained eight accessions of wild or other cultivated species. Two accessions of subsp. *andigenum* grouped with the outgroups. The tree placed the cultivated species *S.* × *ajanhuiri* Juz. & Bukasov, *S.* × *curtilobum* Juz. & Bukasov, *S. phureja*, and *S. stenotomum* within the ingroup, and the outgroups in the basal branch but intermixed with two accessions of *S. tuberosum* subsp. *andigenum* and seven other ingroups.

DISCUSSION

Utility of Microsatellites for Classification of *Solanum tuberosum*

High levels of polymorphism and heterozygosity in the microsatellite regions of *S. tuberosum* suggest that microsatellites may be a useful tool to detect genetic differences between closely related taxa. In this study, the IAM with neighbor-joining and least-squares distinguished subsp. *tuberosum* from subsp. *andigenum*. Subspecies *andigenum* is intermixed with other cultivated species and members of their progenitors in the *S. brevicaule* complex, concordant with nuclear RFLP and RAPD results of Miller and Spooner (1999).

Although microsatellites are claimed to be useful for species-level phylogenies (Takezaki and Nei, 1996), it appears there is no consensus among researchers as to which evolutionary model is most appropriate for reconstructing phylogenies based on microsatellite data (Feldman et al., 1999; Goldstein and Pollock, 1997). Therefore, trees of *S. tuberosum* were constructed using both the SMM model ($\partial\mu^2$) (Goldstein et al., 1995) and the IAM model of Nei (1972). Both models failed to absolutely distinguish subsp. *andigenum* from subsp. *tuberosum*, or from the other cultivated species. Neither method will clearly separate subsp. *andigenum* from some of its ingroup relatives in the *S. brevicaule* complex and other cultivated species (Grun, 1990; Miller and Spooner, 1999).

It is possible that neither the SMM, which calculates distances based on the number of repeats, nor the IAM, which evaluates all possible alleles, provides the best assessment of the diversity and substructure of *S. tuberosum* (Di Rienzo et al., 1994). The SMM and IAM models were designed for diploids, thus neither method may best estimate the distance between the accessions examined. The microsatellite results (Fig. 2) are not totally congruent with the outgroup relationships of Spooner and Castillo (1997), suggesting that microsatellite data show homoplasy with more distant related taxa. Decreasing utility of microsatellites with increasing taxonomic distance is also clear from the much greater number of null alleles in the outgroup (Table 2), as is common in most other systems studied (Roa et al., 2000). Because the source of the primer sequences is *S. tuberosum* (Milbourne et al., 1998), our ingroup results are more likely to be valid.

Taxonomy and Origins of *Solanum tuberosum*

Various hypotheses have been proposed to explain the origins of the cultivated tetraploid potato. Infinite allele model or neighbor-joining results (Fig. 2) support

the concept that most of the Chilean populations of subsp. *tuberosum* are distinct from subsp. *andigenum*, and other diploid and tetraploid wild and cultivated populations. Subspecies *andigenum* and *tuberosum* are separable but only by using a complex of morphological characters that have overlapping character states (Huamán and Spooner, 2002). They also differ in daylength, adaptation to flowering, and most populations of subsp. *tuberosum* have a cpDNA deletion mutation. Many studies (Grun, 1990; Huamán and Spooner, 2002) suggested that cultivated potatoes are possibly of multiple origins, and that they hybridize with wild species.

The microsatellite data place *S. maglia* with subsp. *tuberosum*, which could be interpreted to support this species as its progenitor, but in agreement with Cribb and Hawkes (1986), Hosaka and Hanneman (1988), and Hawkes (1990), we consider this unlikely. *Solanum maglia* is restricted to coastal Chile, 1000 km north of native *S. tuberosum*, with a single population in the mountains of Argentina, and most populations are sterile triploids (Hawkes, 1990). Identification of extant populations of *S. maglia* in southern Chile by Ugent et al. (1987) are not backed up by voucher specimens of others who have collected potatoes extensively in Chile (Contreras-M, 1987; Spooner et al., 1991). It is possible that *S. maglia* is a diverged population of *S. tuberosum*.

In traditional Andean farming systems, there is ample room for mixing wild and weedy species with cultivated potatoes. Fields of native cultivated potatoes in the Andes are a mixture of various ploides (Ochoa, 1958), and these frequently occur with weedy wild relatives. Farmers select and maintain varieties on factors such as yield, disease resistance, storage longevity, and taste (Brush et al., 1981). As a result, they have developed a large diversity of cultivated varieties that are ecologically versatile (Brush et al., 1981; Zimmerer, 1995). Because of this selection within a loose agroecosystem, the species of Andean potatoes likely cross and form a large, plastic genepool. At the genotype level, there is considerable heterogeneity and endemism of native potatoes (Zimmerer and Douches, 1991), but this variability is difficult to divide into species.

In conclusion, nuclear microsatellites developed for *S. tuberosum* analyzed with IAM or a neighbor-joining model (Fig. 2) support the genetic difference of most landrace populations of subsp. *tuberosum* and *andigenum*. These support their recognition at some classification level.

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