A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping

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The cultivated potato, *Solanum tuberosum*, ultimately traces its origin to Andean and Chilean landraces developed by pre-Colombian cultivators. These Andean landraces exhibit tremendous morphological and genetic diversity, and are distributed throughout the Andes, from western Venezuela to northern Argentina, and in southern Chile. The wild species progenitors of these landraces have long been in dispute, but all hypotheses center on a group of ≈20 morphologically very similar tuber-bearing (*Solanum* section *Petota*) wild taxa referred to as the *S. brevicaule* complex, distributed from central Peru to northern Argentina. We present phylogenetic analyses based on the representative cladistic diversity of 362 individual wild (261) and landrace (98) members of potato (all tuber-bearing) and three outgroup non-tuber-bearing members of *Solanum* section *Etuberosum*, genotyped with 438 robust amplified fragment length polymorphisms. Our analyses are consistent with a hypothesis of a ''northern'' (Peru) and ''southern'' (Bolivia and Argentina) cladistic split for members of the *S. brevicaule* complex, and with the need for considerable reduction of species in the complex. In contrast to all prior hypotheses, our data support a monophyletic origin of the landrace cultivars from the northern component of this complex in Peru, rather than from multiple independent origins from various northern and southern members.

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The origin of crop plants has long fascinated botanists, archaeologists, and sociologists with the following fundamental questions: When, where, how, why, and how many times did crop domestication occur? What are the wild progenitors of these crops? How do crops differ from their progenitors, what selective processes, and how many genetic changes produce these changes? Did crops have single or multiple and separate origins (1–5)? Single (diffusionist) vs. multiple origin (*in situ*) hypotheses of crop origins has long been the subject of debate (6–8). We have used multilocus molecular data from amplified fragment length polymorphisms (AFLPs) to reassess a single vs. multiple origin of landrace cultivars of cultivated potato.

Primitive indigenous cultivated (landrace) potatoes are widely distributed in the Andes from western Venezuela south to northern Argentina, and in Chiloé Island and the adjacent Chonos Archipelago of south-central Chile. The Chilean landraces are secondarily derived from the Andean ones (9), likely after hybridization with the Bolivian and Argentinean species *Solanum tarijense* (10). Potato landraces have been classified into 21 species (11, 12), 7 species (9), 9 species (13, 14), or as a single species, *S. tuberosum*, with eight cultivar groups (15). The landraces are very diverse, with hundreds of clones differing in tuber colors and shapes, and leaf, floral, and growth habit variations. Ploidy levels in cultivated potato range from diploid ($2n = 2x = 24$), to triploid ($2n = 3x = 36$), to tetraploid ($2n = 4x = 48$), to pentaploid ($2n = 5x = 60$). The wild relatives of these landraces (*Solanum* section *Petota*) are all tuber-bearing and include ≈190 wild species that are widely distributed in the Americas from the southwestern United States to southern Chile (16, 17); they possess all ploidy levels of the cultivars, as well as hexaploids ($2n = 6x = 72$).

The wild species progenitors of these Andean landraces have long been in dispute, but all hypotheses center on a group of ≈20 morphologically similar wild species referred to as the *S. brevicaule* complex, distributed from central Peru to northern Argentina (18–22). Members of the complex are morphologically similar to the landraces. Potato domestication from these wild species involved selection for underground characters of shorter stolons, larger tubers, (often) colored and variously shaped tubers, and the reduction of bitter tuber glycoalkaloids; aboveground characters of wild and cultivated species are similar but with cultivated types exhibit high vigor and extensive segregation for flower and foliage traits. The *S. brevicaule* complex includes diploids, tetraploids, and hexaploids. Many members grow as weeds in or adjacent to cultivated potato fields and form crop–weed complexes (19). Morphological data (21) and single- to low-copy nuclear restriction fragment length polymorphism data (22) failed to clearly differentiate wild species in the *S. brevicaule* complex from each other or from most landraces (although the landraces often are taller and more vigorous as a group than the wild species), and the most liberal taxonomic interpretation from these studies was to recognize only three wild taxa: (*i*) the Peruvian populations of the *S. brevicaule* complex, (*ii*) the Bolivian and Argentinean populations of the *S. brevicaule* complex, and (*iii*) *S. oplocense* (Bolivia and Argentina).

Literally all hypotheses have suggested complex hybrid or multiple origins of the cultivars from both northern and southern members of the *S. brevicaule* complex (9, 13–15, 19–21, 23–25). This study investigates these hypotheses through phylogenetic analyses that incorporate the first comprehensive sampling of landraces, the putative progenitors, and outgroups.

Materials and Methods

Plant Materials. We sampled 362 individual wild (261) and landrace (98) members of tuber-bearing relatives of potato (*Solanum* section *Petota*) and three sister group representatives in *Solanum* section *Etuberosum* (*S. etuberosum* and *S. palustre*). These accessions came from the United States Potato Genebank (www.ars-grin.gov/nr6) and the Commonwealth Potato Collection of the Scottish Crop Research Institute (www.scri.ac.uk/cpc). They were identified mostly from living accessions planted at the genebank by visiting potato taxonomists (mainly Jack Hawkes and Carlos Ochoa) over 30 years. The 264 wild species

Abbreviations: AFLP, amplified fragment length polymorphism; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

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accessions are mostly members of the *S. brevicaule* complex and are largely the same ones used in prior morphological (21) and molecular (22) studies of the complex, and are labeled as those species. We included tetraploid *S. stoloniferum* to have a data set comparable to the prior morphological and molecular studies. The members of the *S. brevicaule* complex are so similar that their identities have frequently changed, and we mostly use the identities from these prior studies for consistency. We add additional cultivated accessions and wild species to represent the entire four-clade diversity of section *Petota* from Spooner and Castillo (26), and they included members of the Phureja Group (diploid, Andean), Stenotomum Group (diploid, Andean), Andigenum Group (tetraploid, Andean), and Chilotanum group (tetraploid, lowland Chile). Our study included 230 diploids ($2n = 2x = 24$), 120 tetraploids ($2n = 4x = 48$), and 12 hexaploids ($2n = 6x = 72$). *S. gourlayi* included its diploid and tetraploid cytotypes, and *S. oplocense* included its tetraploid and hexaploid cytotypes. Designations of ploidy and classes of species (cultivated, *S. brevicaule* group north, *S. brevicaule* south, outgroups, and ploidy) are presented in Fig. 1.

AFLP Genotyping. Plants were grown in the glasshouse, and DNA was extracted from frozen plant leaf tissue taken from single plants by using the DNeasy Plant DNA Extraction kit (Qiagen, cat. no. 69181). AFLP assays were performed by using a modification of the protocol of Vos *et al.* (27), using the 6-bp cutting enzymes PstI and EcoRI and the 4-bp cutting enzyme MseI. The six AFLP primer combinations used were EAAC + MCCA, EACA + MCAC, PAC + MACT, PAG + MACC, PAT + MAAC, and PCA + MAGG. PCR reactions were set up on 384-well plates by using a Beckman Biomek 2000 liquid handling device. Electrophoresis was carried out on the Bio-Rad SequiGen GT system on 5% acrylamide and 7 M urea in 1× TBE buffer (100 mM Tris/100 mM boric acid/2 mM EDTA). A dual buffer system of 1× TBE and 1× TBE supplemented with 0.5 M NaOAc was used to create an ionic gradient, which resulted in better separation of the larger fragments. A Promega *fmol* DNA Cycle Sequencing system (Promega Q4100) marker (prepared according to the protocol but using only d/ddT Nucleotide Mix) was run to estimate fragment sizes. Gels were dried onto paper and visualized by exposure to x-ray film (Kodak BIOMAX MR). Gels were scanned by using a standard flat-bed scanner at 300 dpi and 8-bit grayscale format. The TIFF images were then imported into AFLP-QUANTAR software supplied by Keygene (Wageningen, The Netherlands). To enable automatic positioning of marker bands, a standard genotype was run on each gel. The AFLP data matrices are published as supporting information on the PNAS web site.

Phylogenetic Analysis. Phylogenetic reconstructions were performed by using PAUP* 4.0b8 (28), using Wagner parsimony (29). The non-tuber-bearing species *S. etuberosum* and *S. palustre* (section *Etuberosum*) were designated as the outgroup, but members of these species, and clades 1, 2, and 3 of Spooner and Castillo (26), fall at the base of the tree and are listed as outgroups in Fig. 1; all remaining wild and cultivated species are members of clade 4. To find multiple tree islands, we used a four-step search strategy, modified from Olmstead and Palmer (30). (*i*) One million replicates initially were run by using random order entry starting trees with nearest-neighbor interchange. (*ii*) The shortest trees from this analysis were used individually as starting trees with the tree-bisection-reconnection (TBR) method. (*iii*) The resulting trees were searched with nearest-neighbor interchange, retaining all most parsimonious trees (MULPARS). (*iv*) The resulting trees were searched with TBR and MULPARS. The last two analyses were terminated at 10,000 trees. The resulting trees were used to compute a strict consensus tree. A bootstrap analysis was conducted on 500 replicates with TBR and MULPARS. The above analyses were done twice, once with the entire data set and again only with the diploids.

AFLP data were also analyzed by neighbor-joining using NTSYS-PC^R 2.02k (31). The program SIMQUAL was used to compute similarity matrices using the "Jaccard" option, which ignores shared absent bands, and which is an appropriate algorithm for AFLPs scored as dominant markers.

Concordance Tests Among the Present AFLP Data to the Prior Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), and Morphological Studies. We tested concordance among our new AFLP results to the morphological (21) and single- to low-copy RFLP and RAPD data (22) of the *S. brevicaule* complex. For each data set, we constructed parallel matrices containing accessions in common between studies. We then made pairwise distance matrices for all four data sets. For the AFLP and RAPD data, we used the Jaccard matrix, for the RFLP data we used a simple-matching coefficient, and for the morphological data we used the distance algorithm, all present in NTSYS-PC^R 2.02k (31). We then performed pairwise comparisons of these matrices with the Mantel test (32) as performed in NTSYS-PC^R. This statistic varies from 0 (no correspondence of matrices) to 1 (perfect correspondence).

Results and Discussion

Cladistic Results. The six AFLP primer combinations produced 438 characters of which 3.6% of the data matrix had missing values, caused by occasional failed reactions or faint bands. Wagner parsimony analysis of all 362 accessions (Fig. 1) produced 10,000 (our designated upper tree limit) most parsimonious 13,672-step trees with a consistency index of 0.033 and a retention index of 0.571, and a rescaled consistency index of 0.019. The topology of the entire data set is very similar to the four clade cladistic structure of Spooner and Castillo (26). *S. etuberosum* and *S. palustre* form a monophyletic basal outgroup, sister to *S. bulbocastanum*, *S. polyadenium*, *S. stenophyllidium*, and *S. tarnii* (clades 1 and 2), sister to *S. acroscopicum*, *S. andreanum*, *S. chilliasense*, *S. pascoense*, and *S. paucissectum* (clade 3); this clade also includes two accessions of *S. acroscopicum* and two accessions of *S. multidissectum*, members of the *S. brevicaule* group. The bootstrap analysis (Fig. 1) showed >50% support for the basal clades of sect. *Etuberosum* and clades 1–3, and in some internal branches of clade 4, but poor support (<50%) in the external branches of clade 4. Wagner parsimony analysis of the 230 diploid accessions (data not shown) produced 177 most parsimonious 8,393-step trees with a consistency index of 0.0534, and a retention index of 0.5617 and a rescaled consistency index of 0.0300; bootstrap support was similar to the total taxon tree. The topology of the entire data set and the reduced data set of the diploids differs very little.

The topology of the entire data set is in concordance with the morphological (21), RAPD, and RFLP results (22) in defining a northern (species from Peru, together with *S. achacachense* from northern Bolivia) and southern (species from Bolivia and northern Argentina) clade of the *S. brevicaule* complex. This geographic split does not exactly follow country borders, but very closely so. For example, the northern clade contains *S. achacachense* PI 558032 from the department of La Paz, Bolivia bordering Peru, and the southern clade contains *S. leptophyes* PI 458378 from the department of Puno bordering Bolivia.

Also in concordance with prior results, the AFLP data fail to resolve many species in the complex. Species that fail to form clades in the northern *S. brevicaule* group are *S. abancayense*, *S. bukasovii*, *S. canasense*, *S. leptophyes*, *S. marinasense*, *S. multidissectum*, and *S. multiinterruptum*, whereas *S. candolleanum* and *S. pampasense* form clades. Species that fail to form clades in the southern complex are *S. ambosinum*, *S. brevicaule*,

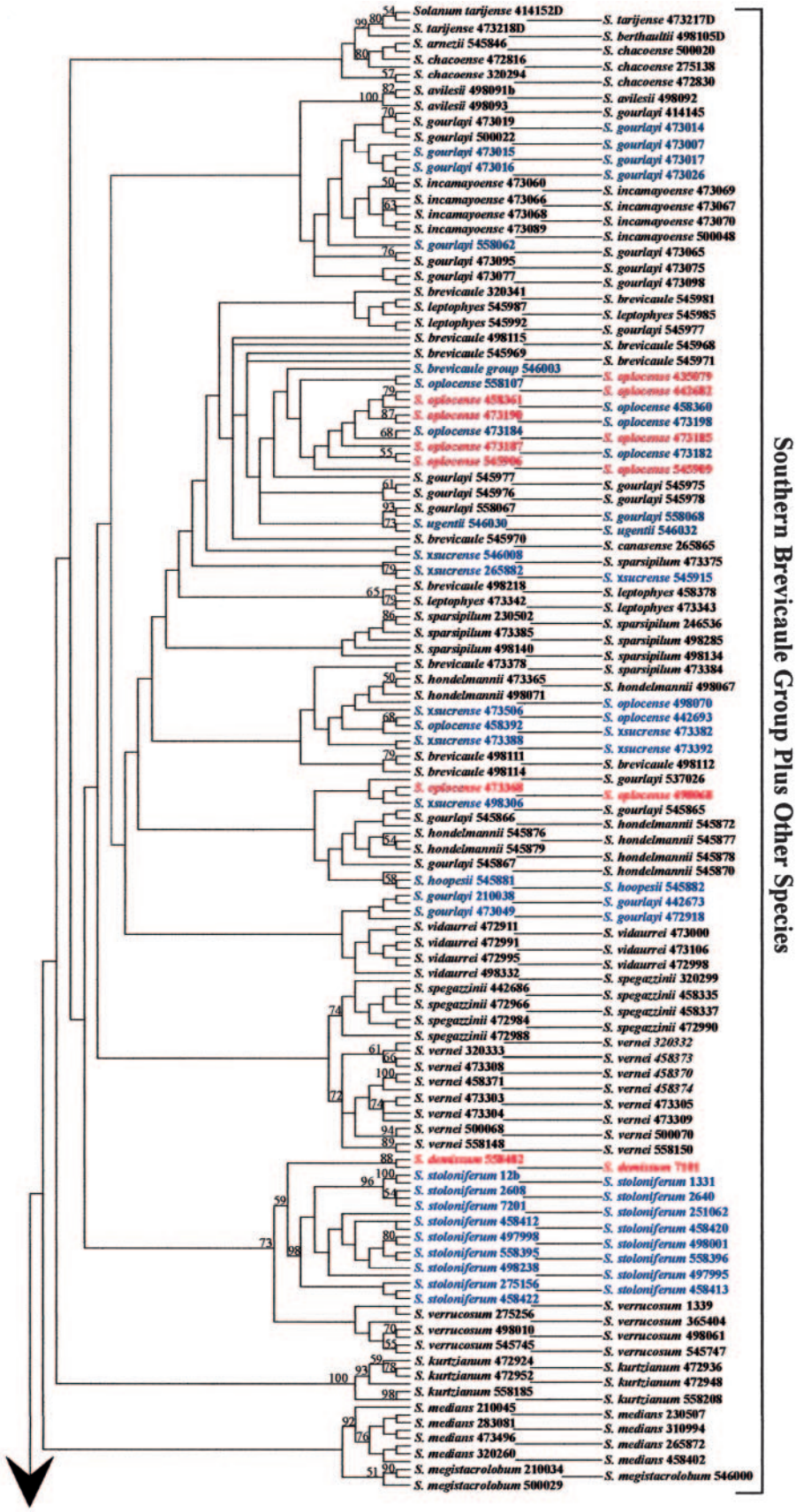
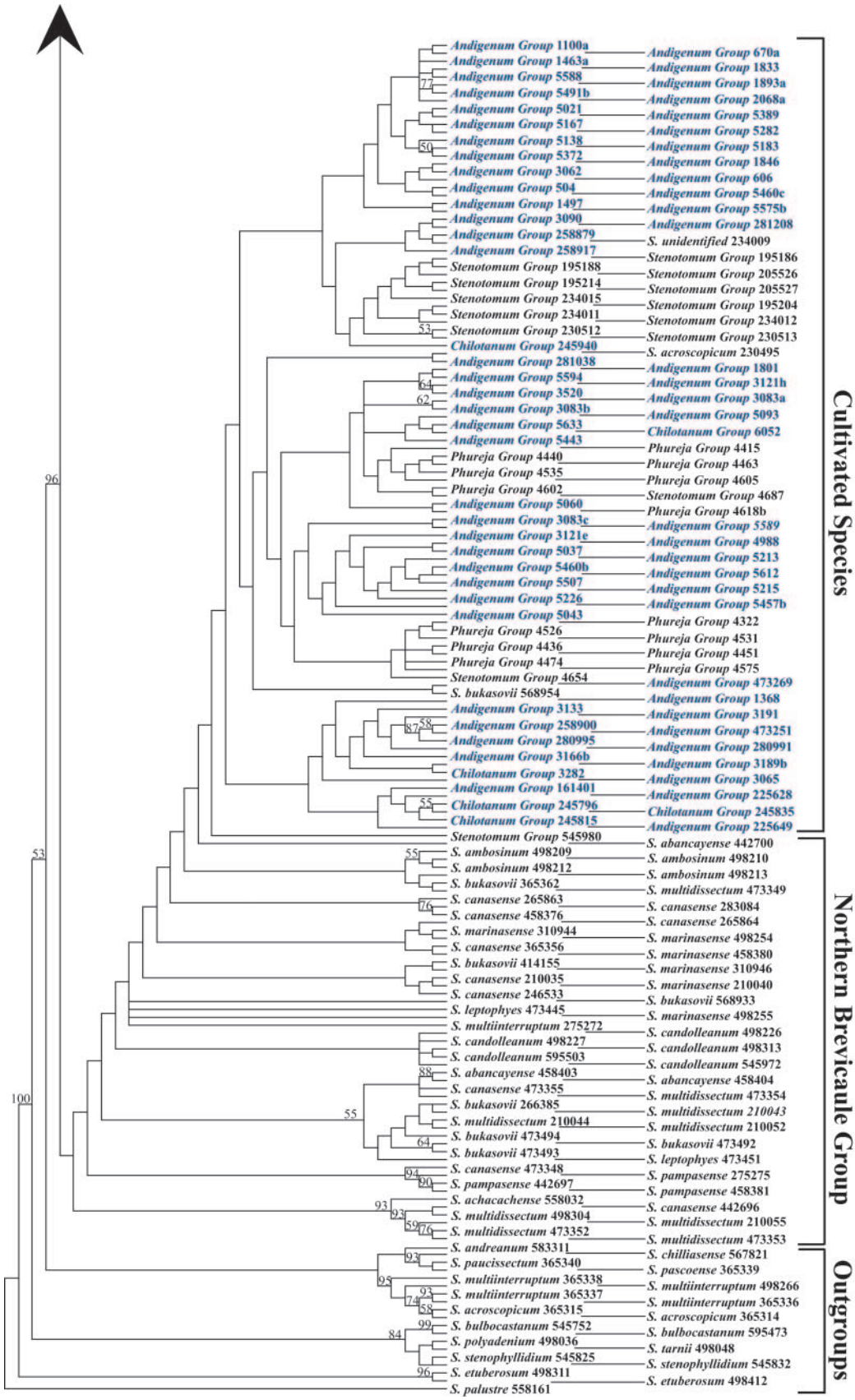


Fig. 1. Strict consensus parsimony cladogram of 10,000 equally parsimonious 13,176-step Wagner trees based on the entire AFLP data set of 362 accessions, with six AFLP primer combinations producing 438 characters. Outgroups consist of three accessions in *Solanum* section *Etuberosum* and clades 1–3 tuber-bearing wild potatoes (section *Petota*) (26). The remaining ingroup consists of members of clade 4, labeled as the northern and southern *S. brevicaule* groups and



cultivated species . The southern *S. brevicaule* group includes species from North and Central America and species from South America that have not traditionally been considered part of this group. For space considerations, the taxa are staggered on the tree. Diploid accessions are colored black, tetraploid accessions are blue, and hexaploid accessions are red.

Table 1. Pairwise comparisons of the present AFLP results to the morphological results (21) and RAPD and single- to low-copy nuclear RFLP results (22) using the same accessions, as performed with the Mantel test (32)

	AFLP	RFLP	RAPD	Morphology
AFLP	—	0.760	0.761	0.182
RFLP	0.740 (166)	—	0.590	0.123
RAPD	0.845 (76)	0.609 (82)	—	0.069
Morphology	0.204 (211)	0.048 (128)	0.121 (69)	—

0 = no correspondence; 1 = perfect correspondence. The results above the diagonal compare the similarity matrices, and those below the diagonal compare the tree topologies transformed to matrix values through cophenetic values (31). The numbers in parentheses below the diagonal indicate the number of accessions common to each comparison.

S. canasense, *S. leptophyes*, *S. oplocense*, *S. sparsipilum*, and *S. sucrense*, whereas *S. avilesii*, *S. hoopesii*, *S. incamayoense*, *S. spegazzinii*, *S. ugentii*, *S. vernei*, and *S. vidaurrei* [diploid] (to the exclusion of one accession of *S. gourlayi* [tetraploid]) form clades.

The AFLP data support a monophyletic origin of all of the cultivars. *S. bukasovii* 568954 and *S. acroscopicum* 230495, however, fall within the cultivated clade. Accession 230495 was labeled in the genebank as *S. acroscopicum* (a diploid species), but chromosome counts show it to be tetraploid and likely to be a misidentified cultivated species. *S. bukasovii* 568954 could be a progenitor or an unrecognized cultivated diploid species.

Hosaka (10) showed that *S. tarijense* was a likely maternal contributor in the origin of landraces of the Chilotanum group, because they share a 241-bp chloroplast deletion. Our examination of three accessions of *S. tarijense*, and the related species *S. arnesii*, *S. berthaultii*, and *S. chacoense*, show them to form a clade, separate from the cultivars.

Phenetic Results. Because AFLPs are dominant and anonymous marker data, a case has been made that they should be analyzed with phenetic methods (33). The neighbor-joining tree (data not shown) outlines nearly the same set of species groups including the northern and southern *S. brevicaule* groups, places the cultivated species as a single group, and places the outgroup distant to the tuber-bearing species. A phenogram could represent the phylogeny when similarities are mainly due to shared derived characteristics (34). In our case, conclusions are the same with cladistic or neighbor-joining procedures, and controversies of proper methods to use are therefore moot.

Concordance of the Present AFLP Data to Prior Morphological and Molecular Data in the *S. brevicaule* Complex. The Mantel tests showed high correlations (r) of the present AFLP results to the prior RAPD and single- to low-copy nuclear RFLP results of the *S. brevicaule* complex (22) ($r = 0.740–0.845$) (Table 1) and much lower correlations to the morphological data ($r = 0.204$). Despite much lower correspondence of AFLP, RFLP, and RAPD results to the morphological results (Table 1), the morphological data still showed a north–south partitioning by a canonical variates analysis (21).

The utility of AFLPs to examine relationships of closely related species has been documented elsewhere. For example, Powell *et al.* (35) showed AFLPs and nuclear RFLPs to be significantly correlated in diversity studies of cultivated soybean (*Glycine max* [L.] Merrill) and its progenitor species *G. soja* Hort.; Milbourne *et al.* (36) showed good correlations between AFLPs and RAPDs in *S. tuberosum*; and Russell *et al.* (37) showed good correlations of AFLPs and nuclear RFLPs in *Hordeum*. Such correlations, and the concordance of AFLP to nuclear RFLP, RAPD, and morphological results reported here, support the utility of AFLPs for examining relationships of *S. tuberosum* and the *S. brevicaule* complex.

Single Domestication for Potato. All cladistic and phenetic analyses, of both the diploids and tetraploids, show all landrace populations to form a monophyletic clade, derived from the northern members of the *S. brevicaule* complex. These *S. brevicaule* northern group member "species" are poorly defined, and ongoing studies may reduce them to a single species, with the earliest valid name of the group being *S. bukasovii*. The conclusions of a single origin of cultivated potato from the northern species of the *S. brevicaule* group differs from all conventional domestication hypotheses (9, 13, 15, 19–21, 23–25) in two fundamental respects: (*i*) a single origin is here supported, rather than a series of multiple independent origins; and (*ii*) the origin is confined to the northern component of the *S. brevicaule* complex, rather than to other southern complex species that are commonly mentioned as progenitors, such as *S. sparsipilum* or *S. vernei* (e.g., refs. 9 and 23). A "single" origin is here supported to mean an origin from a single species, or its progenitor (*S. bukasovii*), in the broad area of southern Peru. Because landrace potatoes are currently spread throughout the Andes and Chile, they clearly were diffused from Peru both north and south, assuming present-day distributions of the original cultivars.

The single origin of potato parallels results suggesting single origins of other crops including barley (38), cassava (39), maize (40), einkorn wheat (41), and emmer wheat (42). This differs from multiple origins of common beans (43), cotton (44), millet (45), rice (46), and squash (47). Allaby and Brown (48) criticize the use of "anonymous" marker data of any type (including AFLPs) to infer single crop origins. Results of computer simulations have led these authors to postulate that monophyletic origins can be erroneously inferred when using dominant marker data analyzed by neighbor-joining methods. They assume that pairs of markers, on average, are unlinked and simulate different scenarios for cereal crops. Authors of some of the original studies have replied, suggesting that the "intrinsic quality" of their data outweighs any doubts through use of simulated data (49). It is clear that further, more sophisticated simulations are required, using information on known marker linkage relationships. Moreover, Allaby and Brown (48) do not comment on the applicability of their studies to predominantly outbreeding, clonally propagated crops, such as potato.

Diamond (50) discusses single vs. multiple crop origins in a geographic perspective. He suggests that crops that spread east and west (as einkorn wheat and emmer wheat in the Fertile Crescent), rather than north and south (as squash and cotton in the Americas), have a competitive advantage in rapid diffusion because they take less time to adapt to new habitats. He further contends that such rapid diffusion preempts adoption of competing crops and favors single crop origins, in contrast to multiple origins of crops spread north and south. His paper was written when maize (51, 52) and potato (present results) were thought to have multiple origins. The widespread north and south diffusion of maize and potatoes, and their monophyletic origin, prompts a reconsideration of his geographic interpretation of single and multiple origins.

Reconsideration of the Taxonomy of the *S. brevicaule* Complex. The data strongly suggest that (*i*) considerable reduction of species is needed in the *S. brevicaule* complex and (*ii*) the complex is polyphyletic. AFLP data suggest that some of the species in the complex, however (mentioned above), may be valid. AFLP data also suggest, as do the RAPD and nuclear RFLP data, that some accessions of *S. multiinterruptum* are part of the distinctive clade 3 (series *Piurana*) (22). Taxonomic changes in species circumscriptions are underway as part of a broader-scale taxonomic

revision of the entire genus *Solanum* (53). Poor support for the traditionally circumscribed *S. brevicaule* complex species suggests that designations of species-specific progenitors of the landrace cultivars with this outdated taxonomy (9, 25) are futile. Rather, our results suggest that the Andean landrace cultivars arose from the northern component of the *S. brevicaule* complex. *S. bukasovii* is the earliest taxonomic name possessing priority as this progenitor.

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