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American Journal of Botany, Vol. 79, No. 4. (Apr., 1992), pp. 467-471.

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American Journal of Botany 79(4): 467–471. 1992.

ALLOZYME VARIATION WITHIN SOLANUM SECT. PETOTA, SER. ETUBEROSA (SOLANACEAE)[1]

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Enzyme electrophoresis was employed to measure genetic variation within and divergence among 32 populations of three species in *Solanum* sect. *Petota* (*S. brevidens, S. etuberosum,* and *S. fernandezianum*). These species are self-compatible, diploid ($2n = 2x = 24$), and members of the monophyletic series *Etuberosa. Solanum etuberosum* is distributed in southern Chile, *S. brevidens* occurs in southern Chile and adjacent southern Argentina, and *S. fernandezianum* is endemic to Masatierra Island in the Juan Fernández Archipelago, 650 km west of continental Chile. Very low levels of observed heterozygosity (0.00–0.04) are found within populations of all three species. Interspecific mean genetic identities between *S. brevidens* and *S. etuberosum* (0.854) were similar to their intraspecific values (0.923, 0.865, respectively), with both species monomorphic for alleles at nine of the 12 loci examined. *Solanum fernandezianum* shows no heterozygosity and is more divergent to both *S. brevidens* (0.780) and *S. etuberosum* (0.698) than either is to each other. The divergence of *S. fernandezianum* to *S. brevidens* and *S. etuberosum* results from novel alleles at two of the 12 isozyme loci; in addition, it possesses only a subset of the variability found in *S. brevidens* and *S. etuberosum* at three other loci.

Solanum ser. *Etuberosa* is composed of morphologically very similar species and is clearly monophyletic. Although members of this series lack tubers, they possess a combination of morphological features that suggests that ser. *Etuberosa* is the outgroup to the tuber-bearing wild relatives of the potato, a hypothesis supported by evidence from chloroplast DNA (Hosaka et al., 1984; Spooner, Anderson, and Jansen, 1990).

According to Hawkes (1990), *Solanum* ser. *Etuberosa* contains five species: *S. brevidens* Philippi (with two subspecies); *S. etuberosum* Lindley; *S. fernandezianum* Philippi; *S. palustre* Poeppig ex Schldl.; and *S. subandinum* F. Meigen. The latter two have been considered as rare or extinct by Correll (1962), Hawkes (1990), and Brücher (1966). Montaldo and Sanz (1962) treat *S. palustre* as a synonym of *S. brevidens.* A taxonomic interpretation of ser. *Etuberosa* by Contreras and Spooner is in progress and will be presented elsewhere. The present analysis will consider *S. brevidens* (without regard to subspecies), *S. etuberosum,* and *S. fernandezianum.* These three species are distinguished by differences in leaf shape and pubescence, calyx acumen length, corolla color, and the presence/absence of a seed spot. *Solanum etuberosum* is distributed in southern Chile, *S. brevidens* in southern Chile and adjacent southern Argentina, and *S. fernandezianum* is endemic to Masatierra Island in the Juan Fernández Archipelago, 650 km west of continental Chile (Fig. 1).

Series *Etuberosa* is phylogenetically isolated within sect. *Petota.* Although all three species are believed to differ from each other by structural chromosomal differences, they form a genetically isolated D genome clade (Ramanna and Hermsen, 1981; Spooner, Sytsma, and Conti, 1991). Strong crossing barriers exist between numbers of ser. *Etuberosa* and all other tuber-bearing species, but interspecific hybrids have been obtained with difficulty between *S. etuberosum* and *S. pinnatisectum* Dun., a Mexican diploid tuber-bearing species (Hermsen and Taylor, 1979) *Solanum brevidens, S. etuberosum* and *S. fernandezianum* are self-compatible diploids ($2n = 2x = 24$; Hawkes, 1990).

In the present study, enzyme electrophoresis was used to measure genetic variation within and divergence among *S. brevidens, S. etuberosum,* and *S. fernandezianum.* The authors wanted to investigate their interspecific relationships by examining isozyme divergence, determine whether the low levels of genetic variation reported for plant populations on oceanic islands are also characteristic of the insular populations of *S. fernandezianum,* estimate time of divergence of *S. fernandezianum* using assumptions of mutation rates and the operation of a molecular clock, and compare the levels of heterozygosity of ser. *Etuberosa* (inbreeding and diploid) to other taxa in *Solanum* sect. *Petota.*

MATERIALS AND METHODS

Seeds of 32 accessions of *S. brevidens, S. etuberosum,* and *S. fernandezianum* were obtained from the Inter-Regional Potato Introduction Station at Sturgeon Bay, Wisconsin (Hanneman and Bamberg, 1986). Twenty-one of these accessions were collected on field expeditions to Chile in 1989 and Argentina in 1990 (Spooner, Clausen, and Contreras, 1991; Spooner, Contreras, and Bamberg,

[1] Received for publication 22 April 1991; revision accepted 10 December 1991.

The authors thank Gregory Anderson, Daniel Crawford, Robert Hanneman, Jr., Jonathan Wendel, and Jack Staub for constructive criticisms of the manuscript; John Bamberg for help in obtaining seed from the Inter-Regional Potato Introduction Station; Karen Ludlum for technical assistance; and Kandis Elliot for artwork. Reference to a specific brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

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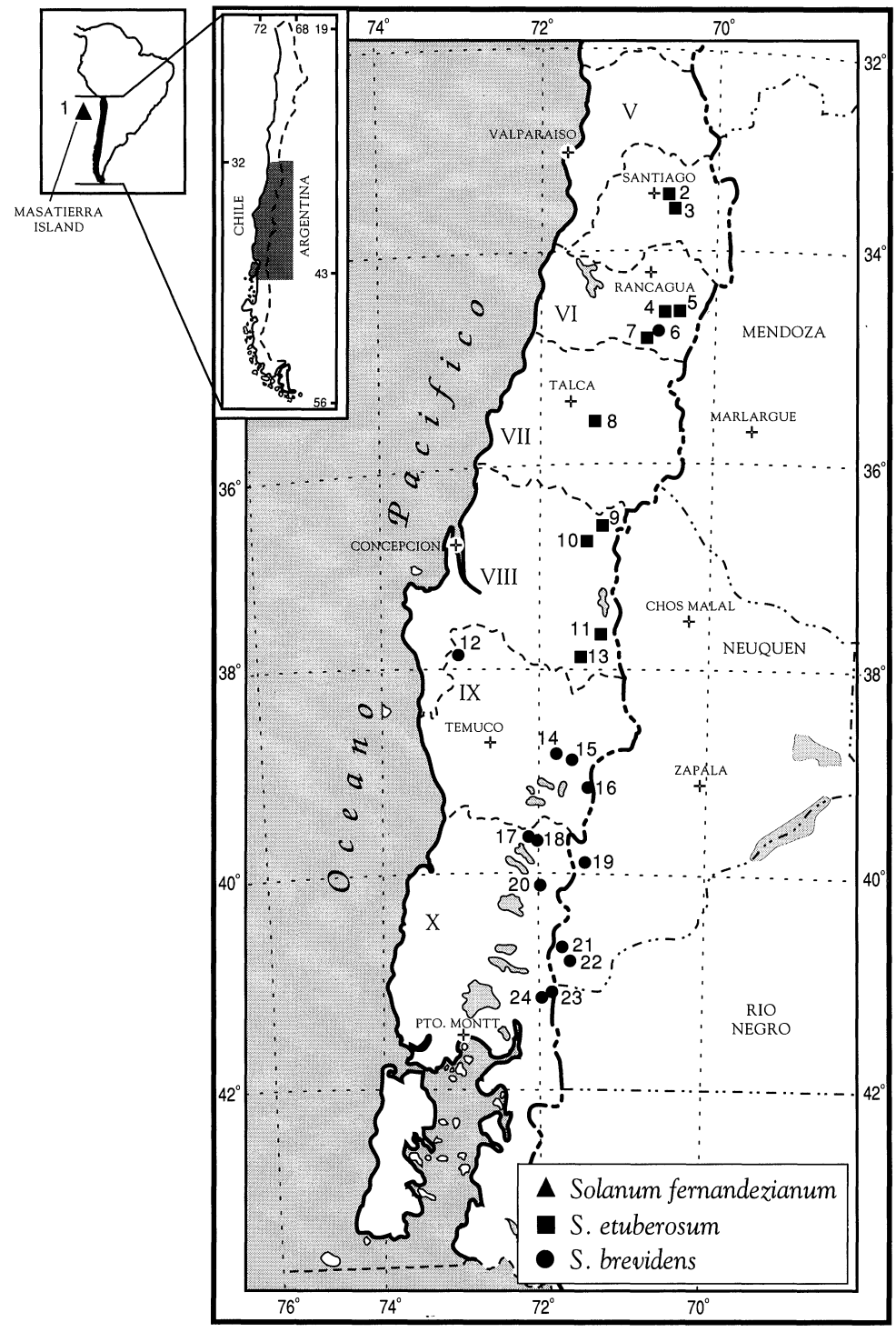

Fig. 1. Distributions of the populations of *Solanum fernandezianum* (triangle), *S. etuberosum* (squares), and *S. brevidens* (circles) examined in this study (see Table 1).

1991), consisted of original seed, and have not yet been assigned plant introduction numbers by the United States Department of Agriculture. The remaining 11 accessions have been through one cycle of seed increase by bulk pollination of 20 individuals. These accessions represent nearly the entire natural range of these three species. They were mapped to 24 geographic areas (Table 1; Fig. 1). Some of the previously collected accessions assigned plant introduction numbers lack precise locality data and were mapped as closely as possible to the recent collections. One leaf was sampled from each of 20 randomly chosen seedlings in each accession.

Tissue was macerated in a 2% glutathione extraction buffer (0.1 M Tris-HCl, pH 7.5), and the crude extract was eluted onto 3 × 8-mm paper wicks (Whatman 3MM®). The wicks were frozen at −20 C overnight if not used

TABLE 1. *Examined populations of* Solanum fernandezianum *(frn),* S. etuberosum *(etb), and* S. brevidens *(brd) (see Fig. 1 for map locations)*

Species, number	Map location	PI[a]	Collector[b]
frn 1	1	320027	*C. Skottsberg* s.n.
frn 2	1	473463	*C. Ochoa 13069*
etb 3	2	—	*S Co 4325*
etb 4	3	—	*S Co 4324*
etb 5	4	—	*S 4473*
etb 6	4	—	*S 4474*
etb 7	5	—	*S Co 4326*
etb 8	7	—	*S Co 4328*
etb 9	8	—	*S Co 4331*
etb 10	9	498311	*Co 1322*
etb 11	10	245924	*D. Correll C143*
etb 12	10	245939	*D. Correll C134*
etb 13	10	498412	*A. Montaldo* s.n.
etb 14	10	—	*S Co 4338*
etb 15	10	—	*S Co 4340*
etb 16	11	—	*S 4490*
etb 17	13	—	*S Co 4349*
brd 18	6	—	*S Co 4329*
brd 19	12	—	*S 4484*
brd 20	14	245763	*D. Correll C14*
brd 21	15	—	*S Co 4392*
brd 22	16	—	*S 4469*
brd 23	17	—	*S Co 4398*
brd 24	18	245764	*D. Correll C15*
brd 25	19	—	*S Cl 4518*
brd 26	20	—	*S Co 4406*
brd 27	21	473401	*Diem* s.n.
brd 28	22	—	*S Cl 4539*
brd 29	23	—	*S 4450*
brd 30	24	—	*S 4451*
brd 31	—[c]	218228	*EBS 338*
brd 32	—[d]	498415	*H. Brücher 81-8*

[a] USDA plant introduction numbers. The accessions without numbers are recent collections that have not yet been assigned PI numbers.

[b] Cl = Andrea Clausen; Co = Andrés Contreras; EBS = Erwin Baur Sortiment Genebank, Germany; S = David Spooner.

[c] This collection was sent to the Inter-Regional Potato Introduction by the Erwin Baur Sortiment Genebank without collector or locality data.

[d] H. Brücher's locality data (Chile, Quetrihue) is confusing. The Peninsula Quetrihue is in the range of *S. brevidens* in Argentina, as are three separate localities spelled Quetrahue in Chile.

immediately. Seven enzyme systems were investigated: malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), phophoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucomutase (PGM), glutamate oxaloacetate transaminase (GOT), and diaphorase (DIA). The crude protein extracts were separated using horizontal starch gel electrophoresis according to Quiros (1981). A 10.5% starch gel (Starchart®) was used to resolve the *Mdh-1, Mdh-2, 6-Pgdh-3, Idh-1,* and *Pgi-1* loci in a histidine-citrate buffer system, pH 5.7; the *Pgm-1, Pgm-2, Got-1, Got-2, Dia-1,* and *Dia-2* loci were resolved in a lithium-borate buffer system, pH 8.3 (Wendel and Weeden, 1989). Formal genetic analyses of these loci previously have been conducted by Quiros and McHale (1985) and Douches and Quiros (1988). Staining protocols followed Vallejos (1983). Leaf samples of greenhouse plants of the potato cultivars Nooksack and Russet Burbank were used as controls to determine the proper allelic designations for the observed bands. Alleles are designated by superscripts. In the context of this analysis homology was assumed between these previously characterized loci in the tuber-bearing species and those of ser. *Etuberosa.*

TABLE 2. *Nei diversity statistics for* Solanum fernandezianum, S. etuberosum, *and* S. brevidens, *based on 12 isozyme loci*

Species	Number of populations	Total diversity H_t	Within population diversity H_s	Between population diversity D_{st}
S. fernandezianum	2	0.000	0.000	0.000
S. etuberosum	15	0.128	0.003	0.125
S. brevidens	15	0.078	0.006	0.072
Total	32	0.143	0.004	0.138

Allelic frequencies were estimated for each population, and these were used to calculate genetic identities for pairwise comparisons of each population (Nei, 1972) and gene diversity statistics (Nei, 1973) using a program written for a personal computer by K. Ritland, University of Toronto.

RESULTS

Allozyme polymorphisms—Nineteen presumptive alleles, encoded by 12 enzyme loci, were identified. Seven loci (*Dia-1, Got-2, Idh-1, Mdh-1, Mdh-2, 6-Pgdh-3, Pgi-1*) were invariant in all populations examined. Two loci (*Got-1* and *Pgm-1*) distinguished *S. fernandezianum* (fixed for *Got-1*^{1} and *Pgm-1*^{2}) from *S. brevidens* and *S. etuberosum* (both fixed for *Got-1*^{2} and *Pgm-1*^{3}). The remaining three loci (*6-Pgdh-1, Pgm-2, Dia-2*) were polymorphic in *S. brevidens* and *S. etuberosum* and monomorphic in *S. fernandezianum.* Intralocus polymorphism occurred in only four of the 32 accessions examined (etb 10 = 0.042, brd 26 = 0.015, brd 29 = 0.040, brd 31 = 0.040). The *Pgm-2*^{2} allozyme was unique to *S. brevidens,* and the *Pgm-2*^{4} allozyme was unique to *S. etuberosum,* but all three species possessed the *Pgm-2*^{3} allozyme. Additionally, *S. etuberosum* had a unique allele (*Dia-2*^{3}) that was found in two of the 15 populations examined. Except for *S. fernandezianum,* the variable alleles exhibited no species-specific or geographic patterns.

Genetic statistics—Within population diversity (Table 2) was very low for all three species (0.000–0.006). Total gene diversity for the entire ser. *Etuberosa* was low (0.143). Most of this diversity was partitioned between, rather than within populations. Intraspecific mean genetic identities ranged from 1.000 in *S. fernandezianum* (complete identity) to 0.923 in *S. brevidens* to 0.865 in *S. etuberosum.* Interspecific mean genetic identities between *S. brevidens* and *S. etuberosum* (0.854) were similar to their intraspecific values (0.865, 0.923). *Solanum fernandezianum* was more divergent from both *S. brevidens* (0.780) and *S. etuberosum* (0.698).

DISCUSSION

Interspecific relationships within Solanum ser. Etuberosa—*Solanum brevidens* and *S. etuberosum* have similar intra- and interspecific mean genetic identities. No species-specific pattern can distinguish either of these taxa from each other, unlike *S. fernandezianum,* which is dis-

tinguished from both of them by two unique alleles (*Got-1*[1] and *Pgm-1*[2]). The relatively young age of Masatierra Island (4 million years, Stuessy et al., 1984), combined with its small size and isolation suggests that *S. fernandezianum* is derived from *S. brevidens* or *S. etuberosum* (or their relatives), rather than the reverse, but the two unique alleles of *S. fernandezianum* are autapomorphies. Thus, the allozyme data are uninformative for elucidating phylogenetic relationships among the species and simply indicate that they are closely related. The partitioning of the variability of *S. brevidens* and *S. etuberosum* between rather than among populations is a common pattern found in inbreeding species (Crawford, 1990).

Allozyme variation of the insular endemic S. fernandezianum—If *S. fernandezianum* is derived from *S. brevidens* or *S. etuberosum,* total allozyme diversity has decreased and two new allozyme loci have evolved. Witter and Carr (1988) suggest that founder events associated with the introduction of ancestral species from mainland source areas can greatly reduce allozymic variation and can allow for the rapid fixation of mutant alleles. Because only two populations of *S. fernandezianum* were available for analysis, its complete lack of genetic diversity relative to *S. etuberosum* and *S. brevidens* (Table 2) may be misleading, and more extensive collections of *S. fernandezianum* are needed to answer questions about its lack of genetic diversity. If *S. fernandezianum* were derived from one of the mainland species, its reduced genetic diversity and/or fixation of new alleles relative to these species would conform to patterns seen in most other groups on oceanic islands (Helenurm and Ganders, 1985; Lowrey and Crawford, 1985; Crawford, Stuessy, and Silva, 1987; Witter and Carr, 1988; Crawford et al., 1990; Inoue and Kawahara, 1990; Wendel and Percival, 1990).

Time of divergence of S. fenandezianum—Because Stuessy et al. (1984) data Masatierra Island at approximately 4 million years old, the maximum time of divergence of this species is known. Divergence times have been estimated given certain assumptions about mutation rates and the operation of a molecular clock (Prager, Fowler, and Wilson, 1976; Ledig and Conkle, 1983; Nei, 1987). The most widely employed method using allozyme data is that of Nei (1987; see Wendel and Percival, 1990 for an application): Time (t) = D/2a, where D is the standard genetic distance and a is the substitution rate per locus per year (approximately 10^{-7} per locus per year). Assuming *S. brevidens* is the progenitor to *S. fernandezianum,* this model would date the divergence time of *S. fernandezianum* at 1.1 million years ($D = 0.220$; $t = [5 \times 10^6]D$).

Comparison of heterozygosity of ser. Etuberosa to wild species within Solanum sect. Petota—Douches et al. (1989) sampled the allelic diversity of *Solanum* sect. *Petota* with 43 accessions representative of the diversity in the group. The average (range) heterozygosity statistics were: diploid outcrossers, 34 accessions tested = 0.157 (0.048–0.318); disomic polyploids, five accessions tested, = 0.302 (0.265–0.350); polysomic polyploids, four accessions tested = 0.225 (0.176–0.262). The within population heterozygosity present in ser. *Etuberosa* (0.00–0.04) is the lowest yet observed in *Solanum* sect. *Petota* and suggests that inbreeding greatly lowers the genetic variability in *Solanum* sect. *Petota.* This effect of the breeding system on heterozygosity is in accordance with patterns well established in plants (Crawford, 1990; Hamrick and Godt, 1990).

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