HIGH LEVELS OF INTERSPECIFIC HYBRIDIZATION BETWEEN *SOLANUM SPARSIPILUM* AND *S. STENOTOMUM* IN EXPERIMENTAL PLOTS IN THE ANDES

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Abstract

Five experimental plots of 3 x 8 m containing 80 plants each, 60 of *Solanum stenotomum* (12 clones) and 20 of *S. sparsipilum* (unknown number of clones) interspaced at random, were established at the Granja K'ayra in Cuzco, Perú. The plantings were intended to mimic small fields of traditional farmers on low fertility soil and steep terrain. Plants of each species were distinguished from another by at least four isozyme loci and by stem pigmentation. Ninety five percent of the seedlings grown from either open-pollinated seed of *S. stenotomum* or *S. sparsipilum* were found to be interspecific hybrids. That the majority of the open-pollinated seed were of hybrid origin suggests that gene flow between weedy and diploid cultivated species is important in generating new landraces of potatoes in the Andes.

Compendio

En la Granja K'ayra, Cuzco, Perú, se establecieron cinco parcelas experimentales de 3 x 8 m conteniendo 80 plantas cada una, 60 de *Solanum stentomum* (12 clones) y 20 de *S. sparsipilum* (número desconocido de clones) interespaciadas al azar. Las siembras intentaron duplicar las condiciones de los pequeños campos de agricultores tradicionales, sobre suelos de baja fertilidad y topografia escarpada. Las plantas de cada especie se distinguieron unas de las otras en por lo menos cuatro loci de isoenzimas así como por la pigmentación del tallo. Se encontró que noventa y nueve por ciento de las plántulas obtenidas de semilla de polinización libre tanto de *S. stenotomum* como de *S. sparsipilum* eran híbridos interespecíficos. El hecho que la mayoría de la semilla de polinización libre fuera de origen híbrido sugiere que el intercambio de genes entre especies no cultivadas y especies cultivadas

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Introduction

Biosystematic, genetic and molecular evidence indicate that the Andean cultivated tetraploid *Solanum tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes has arisen many times by hybridization and introgression involving wild and cultivated diploid species (2,7,12). Hawkes (4) identified the species *S. stenotomum* Juz. et Buk. and *S. sparsipilum* (Bitt.) Juz. et Buk. as the most likely ancestors of *S. tuberosum* ssp. *andigena*. The former is a diploid cultigen restricted to the Andean region, while *S. sparsipilum* is an aggressive diploid weed that occurs in and around the cultivated potato fields of Perú and Bolivia (2,13). The biosystematic work of Ugent (13) suggests that the extensive polymorphism of *S. sparsipilum* derives from continuous hybridization of diploid cultivated species with several related diploid wild species. This process may have resulted in stabilization of a large number of diploid hybrid variants, all pooled under the species name *S. sparsipilum*. Moreover, Ugent (13) suggested that *S. sparsipilum* plays an active role in development of new cultivated diploid potatoes by serving as a genetic bridge, allowing the flow of genes from wild species. This constant influx of genes increases the variability of the gene pool, thus permitting constant selection by man for improved cultivars.

To test Ugent's hypothesis concerning the role of *S. sparsipilum* as a genetic bridge, we planted *S. stenotomum* (2x) and *S. sparsipilum* in experimental fields designed to mimic traditional farmers' fields and then determined the level of interspecific hybridization between them.

Materials and Methods

Five experimental plots of 3 x 8 m and located approximately 250 m from one another were established in Granja K'ayra, Cuzco, Perú at 3300 m altitude (13°35'S, 70°51'W). Each plot contained 80 plants, 60 of *S. stenotomum* (stn) (8 clones) and 20 of *S. sparsipilum* (spl) (number of clones unknown), interspaced at random in rows approximately 1 m apart. Species abbreviations follow the recommendations proposed by Huaman and Ross (8). These accessions represented a small part of the diversity in the species. The stn accessions were supplied by Centro de Investigaciones de Cultivos Andinos, Cuzco. The spl accessions were supplied by the International Potato Center, Lima. Plantings were intended to duplicate small subsistence fields on steep terrain commonly cropped with potatoes by Andean traditional farmers. The nearest cultivated potato fields were at least 1 km away, and no populations of wild potato species were found in the immediate vicinity.

One plot (#3) was used for controlled hand pollinations. For each species, pollen was collected from numerous individuals by vibrating mature anthers with a tuning fork. This pollen was then used to pollinate emasculated flowers. Hand pollinations were performed within 3 hrs. of pollen collection, and the flowers were covered immediately with paper bags to prevent open pollination. Intra- and interspecific control crosses were performed in this fashion. Once berries began to form, the paper bags were replaced with mesh bags.

Berries from open pollination, in the other four plots, were placed in mesh bags as each inflorescence matured.

All plants of both species were harvested at the end of the growing season. Seeds from a total of 18 plants of three stn clones and 7 plants of spl were sampled for the determination (Tables 2 and 3). Selection of stn clones 2, 4 and 8 was based on seed availability from control crosses. After extraction, the seed was treated with 1000 ppm of gibberelic acid to break dormancy. Seedlings were then grown under standard greenhouse conditions.

The phenotype of each seedling was determined for at least two enzyme loci by starch gel electrophoresis of young, healthy leaf tissue (3,12). The two species included in the plots had electrophoretic phenotypes that differentiated seedlings derived from either intra- or interspecific crosses. The diagnostic loci were *Mdh-1, Prx-2, Got-1* and *Got-2*, which were homozygous for different allozymes in the plants sampled from both species. Thus, interspecific hybrids were identified by presence of heterozygous genotypes for the isozyme loci (Fig. 1A). The inheritance of these isozymes has been reported elsewhere (3,12). In addition to the enzyme loci, it was possible to identify interspecific hybrid seedlings from open-pollinated spl seed by the presence of purple pigmentation in the hypocotyl and leaf nodes. After finding perfect association between lack of pigmentation and presence of spl specific isozyme markers in the first 100 seedlings evaluated, the morphological trait was used to quantify the number of interspecific hybrids for spl open-pollinated progeny. Analysis of variance was employed to compare the level of interspecific hybridization among plots for both species. Percentages were transformed to arcsine values before analysis.

Results and Discussion

Seed Yields from Controlled and Open-Pollinated Plots — Intra- and interspecific control crosses differed markedly in their ability to set berries (Table 1) and to produce viable seed. Stn x stn crosses yielded very few berries as compared to spl x spl and interspecific crosses performed in both directions. While it was not possible to assess berry set in open-pollinated plots, seed set for open-pollinated berries provides a rough point of comparison with control crosses. Open-pollinated berries collected from stn plants yielded

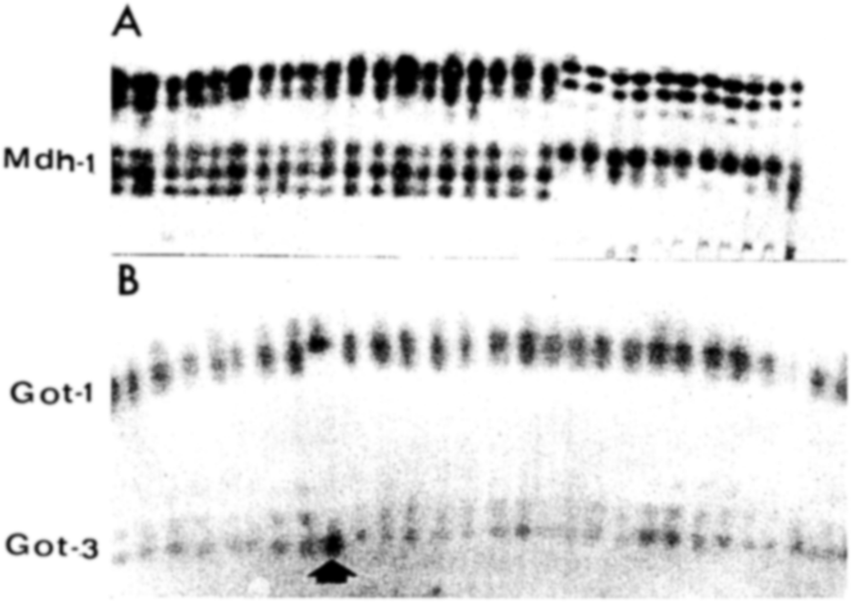

FIG. 1. Zymograms for potato seedlings derived from controlled and open pollinations. Anode is above. A. Discrimination of interspecific from intraspecific seedlings by the locus *Mdh-1*. Interspecific spl x stn hybrids display a three banded heterozygous genotype (lines 1 to 19 from the left), whereas *S. stenotomum* seedlings lack allele *Mdh-1*^{2} of lowest migration (lines 20 to 29). Seedlings derived from controlled crosses. B. Phenotypes of an open-pollinated progeny obtained from a berry of *S. stenotomum*. Only one individual resulted from an stn x stn cross (single bands for both loci, *Got-1* and *Got-3*, arrow). The rest are interspecific hybrids.

TABLE 1. — *Frequency of berry formation for control crosses of* Solanum stenotomum *(stn) and* S. sparsipilum *(spl) at Granja Kayra, Cuzco, Perú.*

Control ♀ × ♂	Number of flowers pollinated	Berries Number set	 (%)
stn × stn	36	3	8.3
spl × spl	59	26	44.1
stn × spl	54	26	48.1
spl × stn	86	82	95.3

2 to 15 viable seeds/berry, whereas berries collected from spl plants yielded between 10 and 450 viable seeds/berry. Most of the spl seed looked plump and fully developed, whereas the stn seed often was smaller than normal and shrivelled. These observations and treatment of the seeds with gibberelic acid make it unlikely that seed dormancy was a factor influencing seed ger-

mination of the different seed types. Sterility was probably a factor affecting seed yield in some crosses since four of 10 hybrid plants derived from stn open-pollinated berries had mostly nonviable pollen. Sterility in these plants was due to chromosomal aberrations resulting in multivalents in diakinesis and laggards in anaphase I. Thus, the low seed set in the stn clones might be due to accumulation of chromosomal aberrations affecting both male and female fertility and probably also to self-incompatibility. The low seed set of stn plants was reflected in the small number of progeny they generated for analysis of interspecific hybridization (Table 2).

Open-pollinated stn Progenies — The level of interspecific hybridization per plant (average of % interspecific hybridization/berry of all the berries collected in a plant) was not significantly different among plots (95% confidence), indicating that pollinator activity was uniform throughout the experimental fields. The small number of progeny sampled (1 to 13 seedlings per berry) gave a wide frequency range of interspecific hybrids per berry, spanning from zero to 100% (Fig. 2). The lowest values were observed for berries that produced no interspecific hybrids, and this was observed for

TABLE 2. — *Percent interspecific hybridization in open-pollinated progenies obtained from three clones of* S. stenotomum *based on % hybrids/berry.*

Clone	Plant	Number of berries	No. of Seedlings Tested		Interspecific Hybridization (%)
2	1-7	3	16		100
2	2-8	4	5		75
2	2-10	1	3		100
2	4-3	9	16		100
Subtotal		17	40	$\bar{x}$	94 ± 6.2
4	1-9	7	25		98
4	1-10	2	5		88
4	2-1	1	3		100
4	2-2	4	17		88
4	2-4	2	4		100
4	2-7	2	9		75
4	4-1	1	1		100
Subtotal		19	64	$\bar{x}$	93 ± 3.6
8	1-1	2	7		100
8	1-8	3	28		92
8	2-3	1	2		100
8	2-6	2	4		100
8	4-10	1	2		100
8	4-11	1	4		100
8	4-12	1	2		100
Subtotal		11	49	$\bar{x}$	99 ± 1.1
Total		47	153	Combined Average	95 ± 1.99

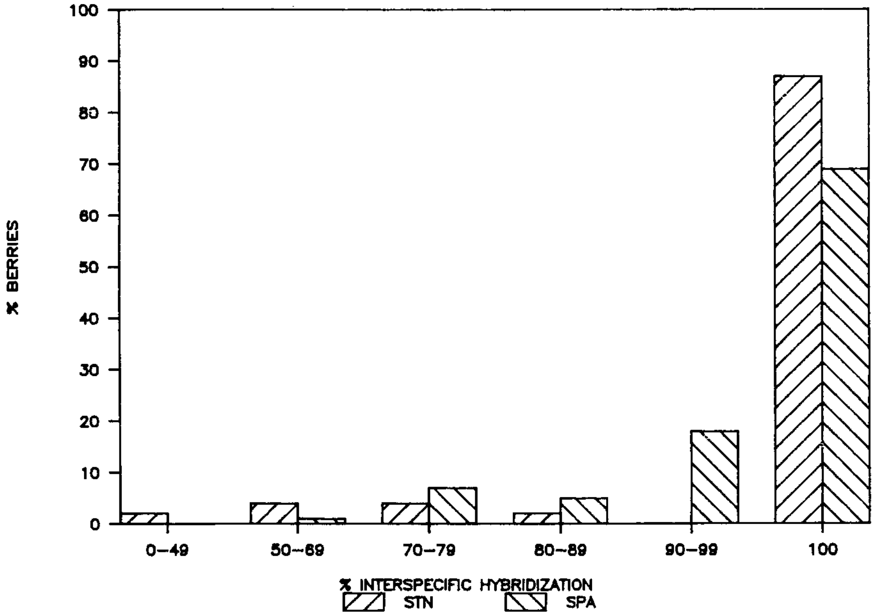

FIG. 2. Frequency distribution of % interspecific hybridization for berries derived from open pollination in *S. stenotomum* and *S. sparsipilum*.

a berry having only a single seedling progency. The next lowest value observed was 50% interspecific hybridization in two berries yielding only two seedlings each. The rest of the berries yielded progenies that were from 70 to 100% interspecific hybrids. The range of interspecific hybridization per plant was from 75 to 100%, with more than half of the plants having only hybrid progency. The overall mean for the stn open-pollinated progenies on a per berry basis was 95% interspecific hybridization (Table 2, Fig. 1B).

Open-pollinated spl Progenies — The same situation observed for the stn open-pollinated progenies was found for progenies derived from open-pollinated berries of spl. However, in this case, seed germination was very good, allowing samples of 18 seedlings per berry on average.

The level of interspecific hybrids per berry, which ranged from 50 to 100% (Fig. 2), was better estimated in spl because of the larger number of progeny (21 to 382 seeds/berry). The range of interspecific hybridization per plant was from 92 to 100% (Table 3). Again, no significant differences for interspecific hybridization were observed among plots (95% confidence). Interestingly, the overall interspecific hybridization mean was 96%, practically the same value observed for the stn progenies. This finding reveals that interspecific hybridization occurs in both directions and at similar intensities, albeit more successfully when spl is the female parent.

TABLE 3. — *Percent interspecific hybridization in open-pollinated progenies from seven* S. sparsipilum *plants based on % hybrids/berry.*

Plant	Number of berries	Progeny	Interspecific Hybridization (%)
1-5	10	133	92.3
1-7	1	21	100.0
4-3	22	318	95.4
4-8	22	382	95.4
5-4	10	335	95.7
5-5	1	23	96.0
5-6	8	95	99.1
Total	74	1307	
Combined Average			96.3 ± 1

Pollination in the experimental plots was mediated by bees of the species *Thygater melanotricha* and *Lonchopria* sp. While *Lonchopria* sp. (formerly *Leioproctus* sp.) has previously been observed pollinating potatoes (9). *T. melanotricha* has not been reported in the literature. Other work performed by us in Tungasuca, Perú (approximately 125 km south of Cuzco in the Rio Vilcanota drainage, altitude 3800 m) (unpublished data), and work by Johns and Keen (9) on the Bolivian altiplano indicate that there are more species of potato pollinators than were first suggested (1a, 3a), the species varying by location and altitude. Our observations make it clear that the high rate of hybridization observed in the present study can be attributed to *T. melanotricha* and *Lonchopria* sp.

Since our plots were isolated from other potato fields, it is safe to assume that the vast majority, if not all, of the pollen interchange took place between the plants in our experiment. It is possible, however, that the level of interspecific hybridization observed was enhanced by our particular planting scheme: interplanting of *S. stenotomum* and *S. sparsipilum* in the same rows. Field studies conducted in Tungasuca, Perú found that spl was relatively common in the disturbed margins of cultivated fields, but was rarely found within the borders of the fields (unpublished data). This evidence suggests that natural hybridization of spl and stn will tend to take place near the borders of cultivated potato fields and with decreasing freqency toward the center of fields. This stands in contrast to work on *S. ajanhuiri* (9,10) where hybrid swarms of *S. ajanhuiri* (weed) were found growing in potato fields with *S. ajanhuiri* (crop) and *S. stenotomum.*

In addition to the dilution of hybridization by the presence of spl primarily in field borders, further dilution would result in farmers' fields since many other species of potatoes would usually be planted with *S. stenotomum* (1,11). It is expected that the probability of pollinators mediating a spl x stn hybridization is in inverse proportion to the number of non-*S. stenoto-*

mum potatoes planted in fields, assuming a haphazard planting regime for various potato species and cultivars (1). However, it is possible that spl will also hybridize in these fields to other related cultivated species. The precise nature of crop/weed hybridization is undoubtedly a function of the ecology of the particular species involved, their pollination biology and the cropping system in use, so field studies of naturally occuring *S. sparsipilum/S. stenotomum* complexes are called for to fully elucidate where hybrids occur and in what quantity. Examples of natural hybrids among other species have been reported by Hawkes and Hjerting (5), Johns and Keen (9) and Johns et al. (10).

Ugent (14) suggested that introgression in the opposite direction occurs from *S. tuberosum* to wild species via *S. sparsipilum,* allowing the establishment of newly generated recombinants which undergo additional cycles of natural hybridization with cultivated potatoes. Since these species share similar natural environments, it is unlikely that ecological barriers will prevent the survival of their natural hybrids (5). The high level of variability generated in the diploid gene pool by interspecific hybridization is likely to cause the influx of meiotic mutant genes determining 2n or unreduced gamete formation, which will eventually result in polyploidization (6). This would permit the establishment of highly polymorphic tetraploid potatoes such as *S. tuberosum* ssp. *andigena.* This hypothesis is consistent with chloroplast DNA data (7) indicating that cultivated tetraploid potatoes of the Andean region arose from cultivated diploid × wild species hybridizations taking place in either direction. The high frequency of interspecific hybridization observed in the present study between spl and stn demonstrates that gene exchange between these two species is a common event when they grow in close proximity to one another and provides experimental evidence supporting Ugent's hypothesis (5,13). The high level of hybridization reported here probably plays an important role in generating new variability, thus helping to account for the large number of varieties (landraces) observed in the Andean region.

Although our study was confined to only two species of potato, interbreeding is likely to occur between a larger number of cultivated and wild species (9,10) which are commonly found in Andean fields. Additional in situ studies are needed to determine the level of interploidy hybridization and to elucidate the natural origins of triploid and pentaploid species.

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