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Genetic and Biosystematic Studies on Two New Sibling Species of *Lycopersicon* from Interandean Perú

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Summary. All available accessions of the entity previously named *"L. minutum"* and described by Chmielewski were investigated biosystematically. All such lines can be unequivocally classified into two elements that are morphologically distinguished, chiefly by differences in flower size and exsertion of the stigma. The stigmas of the large-flowered type are strongly exserted; those of the smaller, slightly or not at all. Both forms are sporadically dispersed in the central and northern Peruvian Andes, east of the continental divide. They are sympatric and often cohabit in the Apurimac-Ayacucho-Cuzco region. Despite this overlap of ranges and intermingling, intermediate types have not been found in nature, although they can be produced experimentally by reciprocal crosses between the two forms. The F_1 hybrids are highly fertile, but seeds produced by self-pollination germinate poorly.

Variation at the individual, populational, and higher levels was assessed in progeny tests of wild plants by analysis of 14 enzyme loci, of which eight proved to be polymorphic. The two taxa could be entirely distinguished by alternative alleles of Got-3; perfect agreement was also found in the sympatric region for alleles of Prx-3; a variable degree of differentiation exists at the other polymorphic loci. For the small-flowered taxon, all tested individuals were homozygous, and all members of a single population had identical genotype; furthermore, only limited differences at two loci were found in the eight tested accessions. In contrast, the larger-flowered type exhibits considerable variation in terms of intra- and interpopulational polymorphy, heterozygosity, and other evidence of outcrossing. With the aforementioned exception, all allozymes detected in the smaller-flowered form are known in the larger-flowered type. All evidence from flower morphology and measures of variability consistently alludes to appreciable outcrossing in the latter and strict autogamy in the former. Considerations of all lines of evidence lead to the conclusion that the self-pollinated entity is sufficiently differentiated from the outcrosser to deserve specific status. The former is named *L. parviflorum*, the latter, *L. chmielewskii*, and Latin diagnoses are presented. It is most likely that *L. parviflorum* evolved sympatrically from *L. chmielewskii* by virtue of its acquiring autogamous reproduction -- an isolating mechanism that is apparently reinforced by poor reproductivity of the interspecific hybrids.

Introduction

Since wild species of *Lycopersicon* have been used extensively as sources of germ plasm for breeding improved tomato varieties, much interest attaches to the discovery of new taxa in this genus. The pioneer investigations of Chmielewski and his colleagues (Berger et al. 1966; Chmielewski 1962, 1963, 1965, 1968a,b; Chmielewski and Berger 1962, 1966; Chmielewski et al. 1964; Chmielewski and Rick 1962) on *Lycopersicon* accessions from interandean Perú revealed the existence of a new entity, which was tentatively labeled *'L. minutum'*. Although no formal description and assignment of specific name were made, the evidence from comparative morphology and genetic tests led to the belief that this taxon deserves species status. In the meanwhile, various accessions have been tested for potentially useful germ plasm. From one such accession unusually high soluble solids content of the mature fruit has been bred into large, red-fruited lines of *L. esculentum* (Rick 1974).

Since the time of Chmielewski's researches, new accessions and new experimental data have been obtained to enhance our knowledge about components of the *minutum* complex. Valuable new accessions were provided by several collectors (Tables 1, 2). In October-November 1970, two of us (M.H. and C.M.R.) in the company of Martha Rick were privileged to travel in the *minutum* haunts of the Departments of Ayacucho, Apurimac, and Cuzco, Perú, where we were able to collect new material and observe populations in their native habitat. All available accessions were subsequently grown at Davis for the purposes of morphological comparisons, progeny testing, assays of intra- and interspecific compatibilities, analyses of hybrid derivatives, and diagnosis

Table 1. Summary of Allozymes in Accessions of *L. parviflorum*

Access No.	LA247	735	1132	1319	1321	1322	1326	1329
Site	Chavinillo	Huariaca	Ingenio	Abancay	Curahuasi	Limatambo	Soracata	Yaca
Department	Huánuco	Pasco	Amazonas	Apurimac	Apurimac	Apurimac	Apurimac	Apurimac
Collector*	Ochoa	Smith	Stephens	H.,R.&R.	H.,R.&R.	H.,R.&R.	R.&R.	R.&R.
Collect. No.	1017	None	None	SAL403	SAL405	SAL406	SAL410	SAL413
No. Plants	?	?	?	3	1	1	5	9
Aps-1 †	2	2	2	2	2	2	2	2
Aps-2	n	n	n	n	n	n	n	n
Est-1	4	4	-	4	4	4	4	4
Got-1	1	1	1	1	1	1	1	1
Got-2	3	3	3	3	3	3	3	3
Got-3	3	3	3	3	3	3	3	3
Got-4	1	1	1	1	1	1	1	1
Prx-1	3	3	3	3	3	3	3	3
Prx-2	+	+	+	+	+	+	+	+
Prx-3a	+	+	+	+	+	+	+	+
Prx-3	+	+	+	1	1	1	1	1
Prx-4	6	4	4	-	-	-	4	5
Prx-7a	+	+	+	+	+	+	+	+
Prx-7	1	1	1	1	1	1	1	1

* Collectors: H. - Miguel Holle O., Ochoa - Carlos Ochoa, R.&R. - Charles M. & Martha O. Rick, Smith - Paul G. Smith, Stephens - Stanley G. Stephens.

† Only allele symbols entered in table. A single symbol signifies that all plants in all progenies possessed the same allele.

of enzyme variability. Analysis of enzyme polymorphy was emphasized because many investigations have already demonstrated its usefulness for testing natural relationships between and within closely related species. It is the purpose of this paper to summarize the results of these investigations and to draw certain conclusions therefrom.

Materials and Methods

Information concerning the collector, collector's number, and site of collection for each accession is given in Tables 1 and 2. For our collections, seeds were harvested separately from each plant in the wild and sown separately for purposes of these investigations. For most of the other accessions, information is lacking concerning the size of the wild populations and the number of plants harvested; consequently, our cultures of these accessions are treated as mixtures and assumed to be typical of the parent population. As our tests reveal, the lack of this information is fortunately of minor consequence because, with only one exception, these accessions were of *L. parviflorum*, which is demonstrated to be remarkably uniform within and between populations.

Standard methods were used for growing the cultures and for the hybridization experiments. For reasons of convenience and more precise control of hybridizations, most of the cultures were grown in the greenhouse.

For tests of enzyme variability, we followed standard procedures of horizontal starch-gel electrophoresis (Brewer 1970; Scandalios 1969; Shaw and Prasad 1970). Details concerning our methods are presented elsewhere (Rick, Zobel and Fobes 1974; Rick and Fobes, in press). Isozymes were tested for the four enzymes -- acid phosphatase (Aps), esterase (Est), glutamate oxaloacetate transaminase (Got), and peroxidase (Prx). Vigorous one-month-old seedlings were sampled in these tests, the full array of Est, Got, and Prx isozymes being assayable from root and basal stem tissues, whilst either those tissues or terminal meristems and leaves serve satisfactorily for Aps.

Eight or sixteen plants were tested for each sample; the accession permitting, additional plants were sampled as necessary. Analysis of the variation in such samples allowed determination of the genotype of the parent plant for each isozyme locus. By identifying heterozygotes, the method incontrovertably distinguishes between selfing of a homozygote, selfing of a heterozygote, outcrossing of a homozygote, and, in many instances, outcrossing of a heterozygote. As revealed below, the data are useful for comparing genotypes of the accessions and larger taxonomic categories in respect of the kinds of alleles and extent of variation between and within populations. They also serve as a basis for comparisons with other tomato species.

Table 2. Summary of Allozymes in Accessions of *L. chmielewskii*

Access.No.	LA1028	1306	1316	1317	1318	1325	1327	1330
Site	Casinchihua	Tambo	Ocros	Hda.Pajonal	Auquibamba	Puente Cunyac	Soracata	Hda.Francisco
Department	Apurimac	Ayacucho	Ayacucho	Ayacucho	Apurimac	Apurimac	Apurimac	Apurimac
Collector*	Ugent&Iltis	H.,R.&R.	H.,R.&R.	H.,R.&R.	H.,R.&R.	R.&R.	R.&R.	R.&R.
Coll.No.	832	SAL390	SAL400	SAL401	SAL402	SAL409	SAL411	SAL414
No.Plants	?	7	1	3	6	1	8	1
Aps-1 †	2	2	2	2	2	2	2	2
Aps-2	n	+	+	2+,1n	1n,5(+/n)	3	4+,2n,2(+/n)	n
Est-1	4	4	4	4	4	4	4	4
Got-1	-	1	1	1	3+,2(1),1(+/1)	+	3+,5(1)	1
Got-2	3	3	3	3	3	3	3	3
Got-3	+	+	+	+	+	+	+	+
Got-4	1	1	1	1	1	1	1	1
Prx-1	4	3	3	3	3	3	3	3
Prx-2	+	+	+	+	+	+	+	+
Prx-3a	+	+	1	2(1),1(+/1)	4(1),2(+/1)	1	6+,1(1),1(+/1)	1
Prx-3	+	+	+/1	+	5+,1n	+	4+,4+/n	+
Prx-4	4	4	4	4	5(4),1(4/6)	4	4(4),2(5),2(4/6)	4
Prx-7a	n	5+,2n?	+	+	2+,3n	+	n	-
Prx-7	1	1	1	1	1	1	1	-

* Collectors: H. - Miguel Holle O., Iltis - Hugh H. Iltis, R.& R. - Charles M.& Martha O. Rick, Ugent - Donald Ugent.

† Only allele symbols entered in table. A single symbol signifies that all plants in all progenies possessed the same allele.

Results

Since several lines of evidence indicate the existence of two readily distinguished taxa, it greatly facilitates presentation of our data to label them permanently at this time. Accordingly, we now apply specific names and follow with Latin diagnoses and experimental justification. Inasmuch as the nomen *L. minutum* has heretofore been applied to the entire complex, confusion will be avoided by christening the two components with new epithets. For the large-flowered species we choose the name of *L. chmielewskii* in honor of the scientist who made the major contribution to our knowledge about this complex. The other is named *L. parviflorum* for its salient morphological feature. The two species are described in the following section.

Descriptions

Lycopersicon parviflorum sp. nov. (Fig.1,3).

Planta ramosissima, repenes, herbacea, perennis. Caulis gracilis, internodia 3-5 cm longa. Caules, folia, pedunculi, pedicelli, calycesque dense glanduloso-puberuli pilis multicelularis non ramosis obtecta. Folia characteristice odorata. Folia lata, plana, interrupte pinnatisecta, 6-9 cm in latitudine, 7-10 cm longa, foliola 5 vel 7 mairora, laminis compluribus inter foliola.

Margines foliolorum serrates vel undulati, pseudostipula absens. Folia interim at *L. peruvianum* var. *humifusum* spectans.

Inflorescentia pauciflora [flora 5-8] nonramosa, curta, axes 3-4 cm longae, interim pedicelli basi bracteati. Pedicelli super medium articulati.

Calyx cum 5-lobatus, tepala anguste lanceolata, 4 mm longa. Corolla lutea, stellata, 11-14 mm in diametro, lobis triangularibus, anguste apiculatis. Tuba anteridialis 6 mm longa, antere subsesiles, apices steriles 1 mm longi, nonincurvati.

Stigma admarginem tube anteridialis inserta [rarisime pauce exserta]. Flores inconspiqui.

Bacca globosa, 10-14 mm in diametrio, puberula, mollis, alboviridis [matura] cum 2 virides vel pur-

Fig.1. Representative plant parts of *L. parviflorum*. Note smaller leaves and great reduction of flowers in inflorescences. LA1326 from Soracata (Apurimac), Perú.

pureas lineas, calyx accrescens. Semina parva, 1.5-2.0 × 1.0 mm, late, oblanceolata, purula.

Plant copiously branched, reclining, herbaceous perennial. Stems slender, internodes 3-5 cm long. All vegetative parts, peduncles, pedicels, and calyces densely puberulent, glandular, occasionally with scattered, multicellular unbranched hairs. Foliage with characteristic odor of burned cheese. Leaves broad, flat, interrupted-pinnate, 6-9 cm wide × 7-10 cm long, with 2, occasionally 3, pairs of opposite major segments and variable numbers of small foliolules, margins serrate or undulate, pseudostipules lacking, the leaves in their simplest form resembling those of *L. peruvianum* var. *humifusum*. Inflorescence 5-8 flowered, unbranched, sometimes with tiny bracts at base and subtending pedicels, all parts comparatively short, overall length of axis 3-4 cm. Pedicels with functional articulation 0.3-0.4 the distance from base of flower.

Calyx 5-parted nearly to base, into slender lanceolate segments 4 mm long. Corolla yellow, stellate, 11-14 mm across, deeply divided into elongate triangular lobes with narrow apices. Staminal cone 6 mm long, bottle shaped, the anthers subsessile, their sterile distal portions 1 mm, unbent. Stigma at mouth of anther tube or very slightly exserted. The flowers very inconspicuous in total aspect of plant.

Fruit borne on somewhat elongated peduncle, the berry globose, 10-14 mm diameter, minutely puberulent, soft, whitish-green at maturity with 2 dark green, sometimes purplish, radial lines. Calyx accrescent, becoming rotate at maturity, its segments reaching 8 mm. Seeds small, 1.5-2.0 × 1.0 mm, broadly oblanceolate, covered with pseudohairs.

Collections. In addition to the collections listed in Table 1, the following have been identified from herbarium sheets.

Perú - Amazonas (Prov. Bagua): between Milagro and Amojas, June 27, 1959, *Ferreyra* 13674 (Mus. de Hist. Nat., Lima). Apurimac (Prov. Abancay): Río Pachachaca 20 km n of Abancay, 2,000 m alt, Feb. 9, 1939, *Stork, Horton,* and *Vargas* 10543 (U.S. Nat., Univ. Cal., Berk.); Curahuasi, Dec. 1962, *Iltis* and *Ugent* 739 (Univ. Wis.). Cuzco (Prov. Anta): Limatambo-Puento Cunyac, May 29, 1956, *O. Velarde N.* 1406 (U.S. Nat.). Huánuco (Prov. Dos de Mayo): Chavinillo, 2100 m alt, Mar. 18, 1951, *Ochoa* 1017 (Herb. Ochoa; type specimen); (Prov. Huánuco): Cerro Calavario, July 24, 1948, *Ferreyra* 803 (Mus. de Hist. Nat., Lima); Quebrada de Huertas, 2200-2250 m alt, June 18, 1958, *Ferreyra* 9207 (Mus. de Hist. Nat., Lima).

It should be noted here that the collection site of LA735 is Huariaca instead of San Rafael as specified in Chmielewski's (1968b) research; the site proved to be much closer to Huariaca than San Rafael.

Lycopersicon chmielewskii sp. nov. (Fig.2, 3)

Planta *L. parviflorum,* similis, robustior, internodia 8-12 cm longa. Folia interim cum pseudostipulos, 10-12 × 14-16 cm, foliola 7, lamina plana vel plicata, marginibus undulatis.

Inflorescentia nonramosa vel in partes 2 divisa, 9-12 cm longa. Bracteae et bracteole copiose et *L. parviflori* maiores.

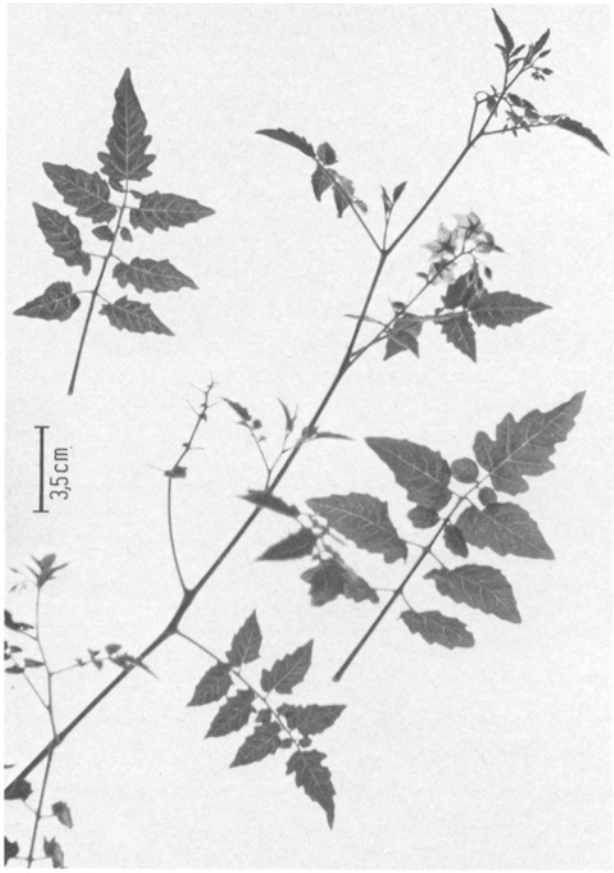


Fig.2. Representative plant parts of *L. chmielewskii*. Note more robust growth, particularly of flowers and inflorescences. LA1306 from Tambo (Ayacucho), Perú.

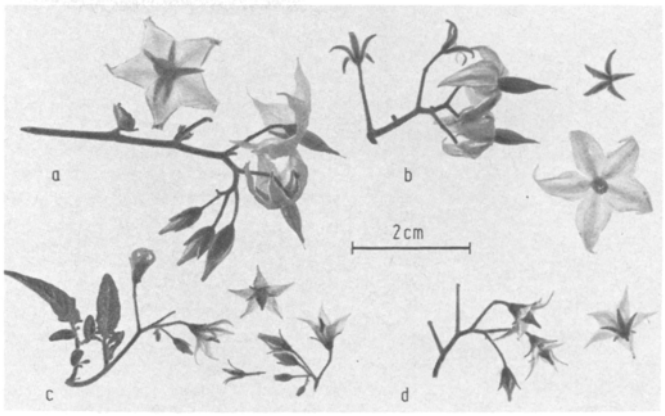


Fig.3. Representative inflorescences of tomato species. a & b - *L. chmielewskii*; c & d - *L. parviflorum*. a - LA1306 (Tambo) Whole inflorescences; b - LA1327 (Soracata) Part of inflorescence; c - LA1326 (Soracata); d - LA1329 (Yaca) Whole inflorescence. Note differences in size of inflorescence and flower, exsertion of stigma.

Tepala 6 mm longa, recurvata. Corolla revurvata, 20-25 mm in diametro, in partes elongatis, triangularibus, apiculatis divisa. Tuba anteridialis 8-9 mm longa, apices steriles 2-3 mm longi. Stigma 1-2 mm exserta. Flores conspiqui.

Bacca luteolo-viridis, calyx accrescens at 20-24 mm.

Generally similar to *L. parviflorum*, but differing in the following respects.

Plant more robust with most parts larger. Internodes 8-12 cm long. Upper stems tending to be flushed with anthocyanin.

Leaves pseudostipulate in certain accessions, 10-12 × 14-16 cm, mostly in 3 pairs of major lateral segments, the laminae flat or plicate with undulate margins.

Inflorescence either unbranched or divided into two cymes, overall length 9-12 cm. Basal and upper bracts more abundant and larger than in *L. parviflorum*.

Calyx segments 6 mm long, recurving. Corolla strongly recurved, 20-25 mm diam, divided halfway to base into elongated triangular segments, apiculate at tips. Staminal cone 8-9 mm long, sterile tips 2-3 mm. Style exserted 1-2 mm. Flowers showy in total aspect of plant.

Fruits ripen to yellowish-green color, calyx enlarging to 20-24 mm diam.

Collections. Table 2 lists all collections known to us. The following is the type collection of this species.

Perú - Apurimac (Prov. Abancay): Casinchihua, above Abancay, Dec. 21, 1962, *Iltis* and *Ugent* 832 (Univ. Wis.; Univ. So. Ill.).

Distribution and Ecology

According to available information, the region of distribution of this complex is limited to interandean Perú, bounded by the Ecuadorean frontier in the north and the Apurimac-Sto. Tomás drainages (Dept. Cuzco) in the south (Fig. 4, 9). No sites are known west of the continental divide nor east of the main cordilleras. *L. parviflorum* appears sporadically throughout this range; *L. chmielewskii* is not known north of Dept. Ayacucho, both are relatively abundant and unquestionably sympatric in the central area of Dept. Apu-

Fig.4. Map of Perú showing sites of the northern collections. Rectangle designates the Ayacucho-Apurimac-Cuzco region shown in larger scale in Fig.9

rimac. The approximate elevation limits of *L. chmielewskii* are 1,500-3,000 m; those of *L. parviflorum*, 1,500-2,500 m.

All collections that we have made of both species were on the lower slopes of the main or tributary drainages in relatively mesic conditions. In general, the soil of the habitats is moist but usually well drained. Both thrive in many types of sites, varying from deep soil to very rocky conditions. The flora is rich and heterogeneous, including many species of low trees, shrubs, and annual and perennial herbs, which were actively growing at the time of collection. Most plants observed of the two tomato species were mature, flowering, and bearing and fruits of various developmental stages. Ripe fruits, as previously observed (Rick 1973), tend to be scarce. In consideration of collectors' observations and the relatively uniform climate, we suspect that flowering continues throughout the year.

Populations tend to be small and scattered in an elongate pattern, parallel to drainages along rock outcroppings and stream banks, generally in inclined situations. *L. chmielewskii* prefers higher, better drained conditions, whilst *L. parviflorum* is more

Fig.5. Photograph taken at Soracata (Apurimac) Perú near the banks of Río Pachachaca, November 8, 1970, illustrating intermingling of both species. A plant of *L. chmielewskii* (LA1327) is perched on the rock in the center (left arrow), whilst branches of a plant of *L. parviflorum* cascade over the flanks of the same rock to the right (right arrow).

apt to occupy lower, moister habitats. These preferences are altogether surprising because we find that *L. parviflorum* is far more sensitive to excessive watering in experimental cultures. The preferences are not so exacting, however, as to separate the species in the sympatric region. On the contrary, we found three situations along Río Pachachaca where their populations overlapped; in fact, plants were in actual contact (Fig.5). In view of the small number of total collections and the relatively brief visit, we suspect that cohabitation is probably not an uncommon phenomenon.

Compatibility and Interspecific Genetcis

Previous research by Chmielewski (1962, 1966) and Chmielewski and Rick (1962) delineated the compatibility relations between *L. parviflorum* and the other tomato species. Our more recent investigations have assayed the same for *L. chmielewskii*. The results of all such tests are summarized in the traditional polygon fashion in Fig.6. Here it can be seen that the crossing reactions of the two species are practically identical: with some minor exceptions, they both behave in essentially the same fashion in crosses with the other species. One highly significant feature of the compatibility patterns is that both are much more closely affiliated with members of the *'esculentum* complex' *(L. esculentum, L. pimpinellifolium, L. cheesmanii, L. hirsutum, Solanum pennellii;* arrayed in the upper part of Fig.6) than with the other greenfruited species *(L. chilense, L. peruvianum).*

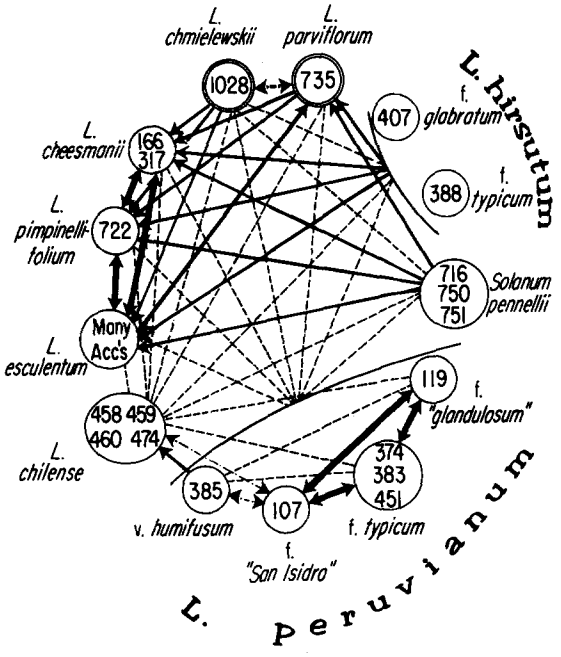


Fig.6. Crossability polygon of the tomato species. Accessions with the same compatibility relations are encircled. Arrows designate direction of compatible crosses; width of lines, degree of compatibility; dashed lines, combinations that fail or will succeed only with special aids; dot-dashed lines, combinations that yield F_1 but for various reasons fail in later generations

Both display the greatest compatibility with the cultivated tomato *(L. esculentum)*, the very closely related *L. pimpinellifolium*, and *L. cheesmanii*, the third colored-fruited species. With other members of the *esculentum* complex, the sibling species display unilateral compatibility and generally reduced seed set. Some degree of differentiation between accessions of *L. parviflorum* is suggested by unilateral vs. complete compatibility with *L. esculentum* and by data presented below.

Of prime interest is the genetic relationship between *L. parviflorum* and *L. chmielewskii*. We have been able to make crosses between the two (LA247 and LA1028, respectively), in reciprocal directions, *albeit* at a considerably reduced seed set. For the cross LA247 (♀) × LA1028 (♂), hybridization was attempted on 32 flowers on two different plants; 18 set fruit. The average seed yield per fruit was 18.5, approximately 25 % of normal. For the reciprocal cross, only two fruits set following hybridization of 28 flowers of two parent plants. The seed count in these fruits approximated 54 % of normal. The results of such crosses are difficult to evaluate because the flower buds of both, but particularly of *L. parviflorum*, require great skill to emasculate without damaging the flower, thereby interfering with fruit set. Additional flowers were pollinated without emasculation in the hope that hybrids might be identified in the progeny. From crosses of both types we have obtained true F_1 hybrids as identified phenotypically.

The F_1 hybrids produced by reciprocal crosses are identical in morphology. The flowers are intermediate in size between those of the parental species - clearly larger than those of *L. parviflorum* and smaller than those of *L. chmielewskii*. They have complete gamete and zygotic fertility as measured by pollen stainability and seed set after self-pollination. Quite unexpected, however, is the severely reduced germination of seeds thereby produced. Despite the application of several kinds of treatments used routinely to break seed dormancy, including sodium hypochlorite, we obtained only a few F_2 plants. Of more than 800 seeds treated in various ways, only 44 germinated and yielded viable seedlings.

It is clear from other experience that determination of genetic isolation should not be based solely on tests of crossability. Even though seemingly good seeds are produced by the hybridizations, other pitfalls in subsequent development and later generations might impede gene exchange between the parent species. An example of this type is found in the experience of Rick (1974) in attempts to breed into *L. esculentum* cultivars the high soluble solids fruit content of *L. chmielewskii*. Good seed yields were consistently obtained from various backcrosses and self-pollinations, revealing the high basic fertility of the derivatives. Yet, much to the consternation of the investigator, low natural fruit set under field conditions persisted, even to late generations. The nature of this unfruitfulness is not known, but it is suspected to relate to a disharmony between parental genotypic control of pollination mechanisms. If the self-pollination mechanisms evolved separately in the two species, it is conceivable that different combinations of genes would result in defective pollination in hybrid derivatives.

That incipient compatibility barriers may also be developing within *L. parviflorum* is suggested by the following experience of E.K. Hybridization between

Table 3. Compatibility Tests between Accessions of *L. parviflorum*

Parental combinations*	Number of flowers pollinated	Number of fruits set	Percentage of flowers that set fruit	Mean number of seeds per fruit
LA735 × LA247	17	5	29	11
LA735 × LA1045	23	4	15	39
LA1045 × LA247	10	0	0	-
LA1045 × LA735	27	0	0	-
LA247 × LA735	19	2	11	33
LA247 × LA1045	4	0	0	-
LA247 Open pol.	-	-	-	42
LA735 Open pol.	-	-	-	78
LA1045 Open pol.	-	-	-	76

* LA247 - Chavinillo; LA735 - Huariaca; LA1045 (Iltis & Ugent #739) - Curahuasi.

three accessions of this species was attempted during the summer under plastic cover. Results of these crosses are compared with the issue of open pollination of each parent in Table 3. None of the crosses yields as well as open pollination, and the least successful hybridizations were LA735 × the other two accessions. In view of the great difficulties in emasculation, these leads should be followed up by more extensive crosses made under various environmental conditions and compared with self-pollination made on emasculated flowers of each accession.

Enzyme Tests

The zymotypes encountered in these sibling species are illustrated in Figs. 7 and 8. Symbols are applied to the alleles following the nomenclatorial rules of the Tomato Genetics Cooperative (Barton et al. 1955; Clayberg et al. 1960; Clayberg et al. 1966). According to this system, the allele present in the standard type of *L. esculentum* is automatically designated normal (+), and variant alleles are given appropriate superscripts. For these series we apply n to the null alleles and numbers to the position shift alleles in order of reporting. The rationale for applying the *esculentum* system to these species is that both can be readily interbred with *L. esculentum* and their genes transferred to it freely. According to all available evidence, these species share the same genetic loci, but differ considerably in allelic constitution.

Of the 14 loci that could be analyzed by our methods, eight were polymorphic; of the 25 total alleles at all loci, 11 are reported here for the first time, the others, in our previous studies (Rick, Zobel and Fobes 1974; Rick and Fobes 1975). The following segregational features have been observed at these loci either in these materials or in the previously studied species. Alleles of Got-1, Prx-1, Prx-3, and Prx-4 are codominant monomers. Dimeric segregation is characteristic of all other Aps and Got loci. Complete dominance of band presence is observed at the other loci. An absolute association has been observed between the band modifications of the Prx-4 moiety in both anodal and cathodal arrays. Normal Mendelian segregation has been encountered for all tested alleles. As in the previously studied species, peroxidase loci exhibit the greatest variability.

Whereas the patterns of banding are generally clear, we cannot always be confident of interpreting the anodal phenotypes of certain Prx-4 alleles. Some preparations suggest that additional alleles may be present. Further testing, particularly of hybrids with *L. esculentum*, should resolve this problem. Until that is done, we prefer to regard the number of alleles conservatively. Whatever the banding patterns, we have found no exception to the complete homozygosity and uniformity within populations of *L. parviflorum* in contrast to considerable variation in all observed populations of *L. chmielewskii*.

The distribution of alleles amongst the various accessions of *L. chmielewskii* and *L. parviflorum* is presented in Tables 1 and 2, respectively. The six loci - Aps-1, Est-1, Got-2, Got-4, Prx-2, and Prx-7

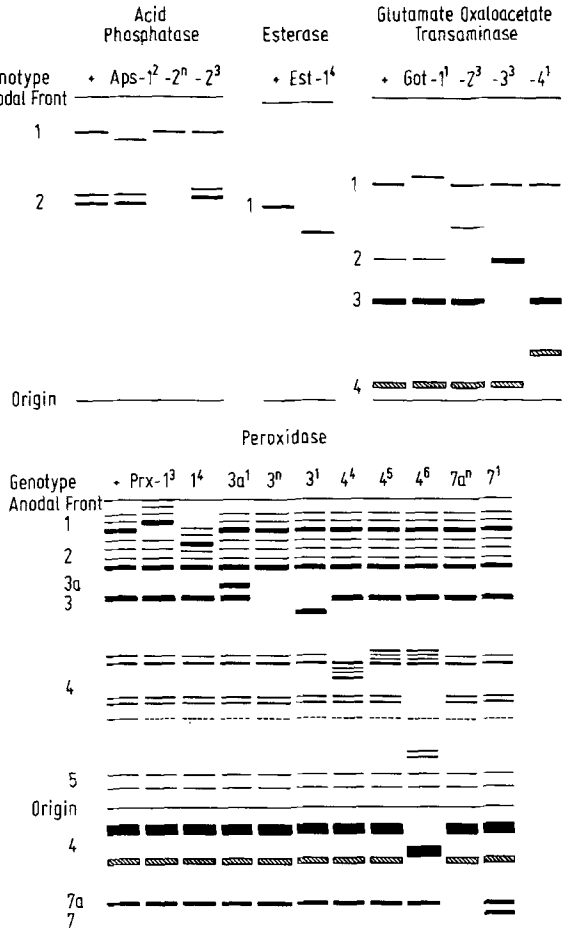


Fig.7. Diagrams of the zymotypes for alleles of four enzyme systems in *L. chmielewskii* and *parviflorum*

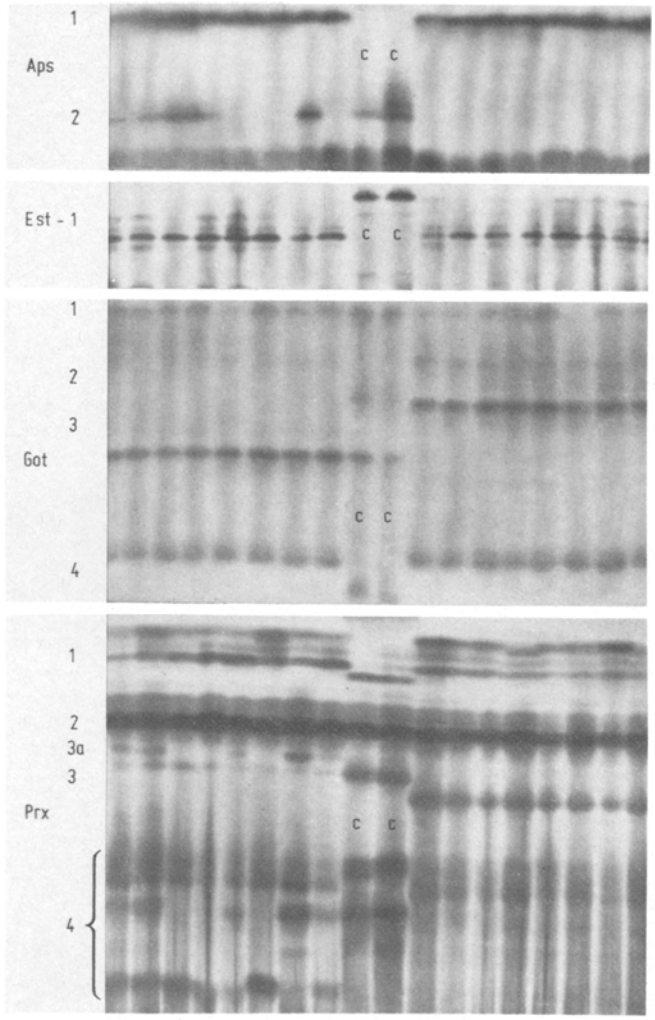


Fig.8. Photographs of starch gels showing representative allozyme phenotypes of the sibling tomato species. In all photographs the two central samples are standard *esculentum* controls (c) flanked by eight samples of *L. chmielewskii* on the left and eight of *L. parviflorum* on the right. All gels are anodal with front at top of each.
Aps - Acid phosphatase. LA1318 (left) Aps-1^2, segregating Aps-$2^+/2^n$; LA1326 (right) Aps-1^2, Aps-2^n.
Est - Esterase. LA1330 (left), LA1328 (right). Genotype of both is Est-1^4.
Got - Glutamate oxaloacetate transaminase. LA1330 (left) Got-1^1, Got-2^3, Got-3^+, Got-4^1; LA1326 (right) Got-1^1, Got-2^3, Got-3^3, Got-4^1. The Got-1 bands of controls (c) are not in the lower position expected of the + allele; evidently migration in that direction was restricted by the anodal front.
Prx - Peroxidase. LA1318 (left) Prx-1^3, segregating Prx-$3a^1/3a^+$, Prx-3^+, segregating Prx-$4^5/4^6$; LA1326 (right) Prx-1^3, Prx-3^1, Prx-4^6. Note homozygosity in all *parviflorum* loci and segregation at certain *chmielewskii* loci

- are monomorphic in both species. For each of the other loci, two or three alleles are known, their distribution amongst populations of the two species being delineated below.

Perfect agreement with the separation of the two species was encountered for Got-3: the normal allele Got-3^+ characterizes *L. chmielewskii*, whilst all tested plants of *L. parviflorum* show the markedly advanced position of the Got-3^3 band. A similar situation exists for Prx-3 in the sympatric region (Dept. Apurimac), where without exception *L. chmielewskii* registers as either Prx-3^+ or Prx-3^n in contrast to the Prx-3^1 of *L. parviflorum*; the presence of Prx-3^1 has been detected in the Ocros (Dept. Ayacucho) accession of *L. chmielewskii*. For each of the remaining six polymorphic loci, *L. chmielewskii* possesses all the known alleles, whilst *L. parviflorum* is monomorphic for all loci excepting Prx-4, for which two variant alleles were found, each in a single population. This degree of variation is not unique to this species because Prx-4 behaves as the most variable locus we have tested in *L. cheesmanii* (Rick and Fobes

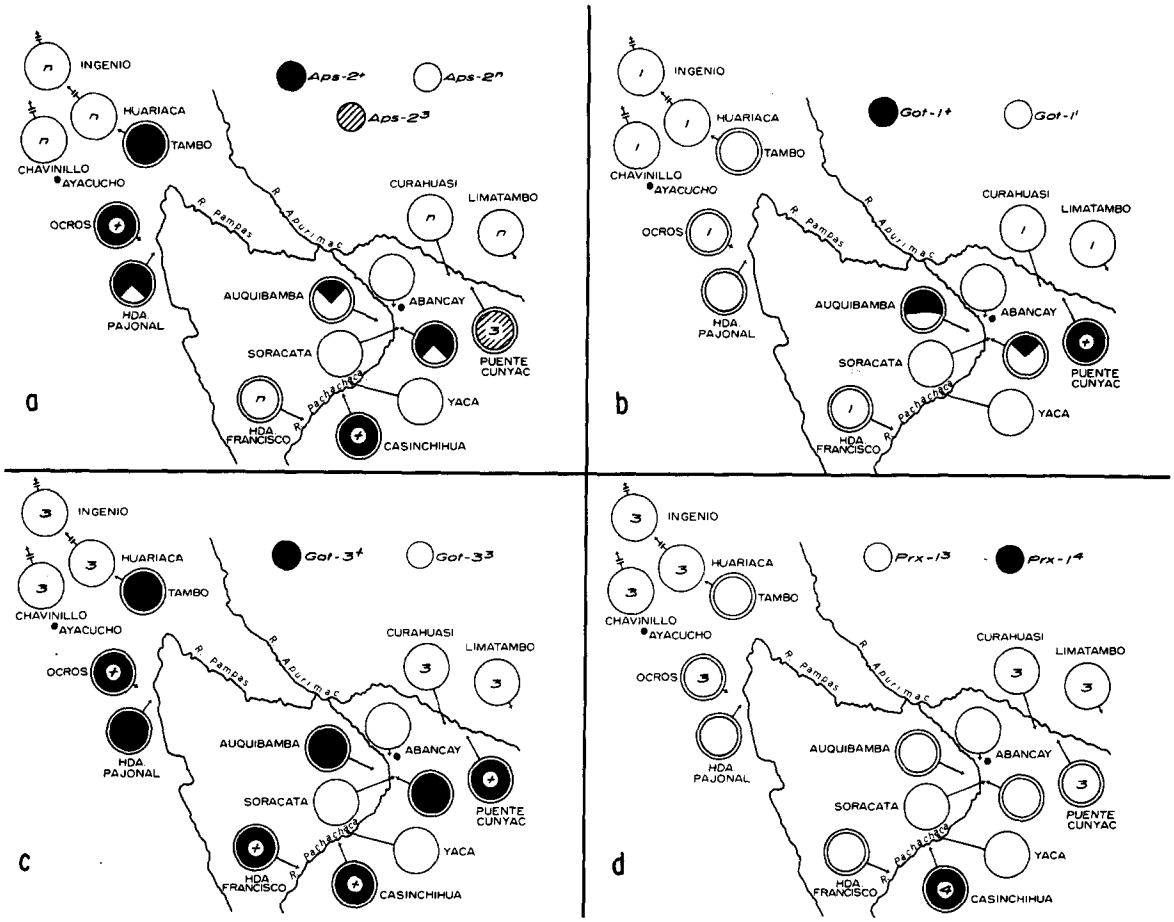


Fig.9. Distribution of allozymes for polymorphic loci in the Apurimac-Ayacucho-Cuzco region of Perú. Sites of the northern collections, which are indicated in Fig.4, are represented by broken arrows. Double circles desig-

1975) and *L. pimpinellifolium* (Rick, Zobel and Fobes 1974, and unpublished).

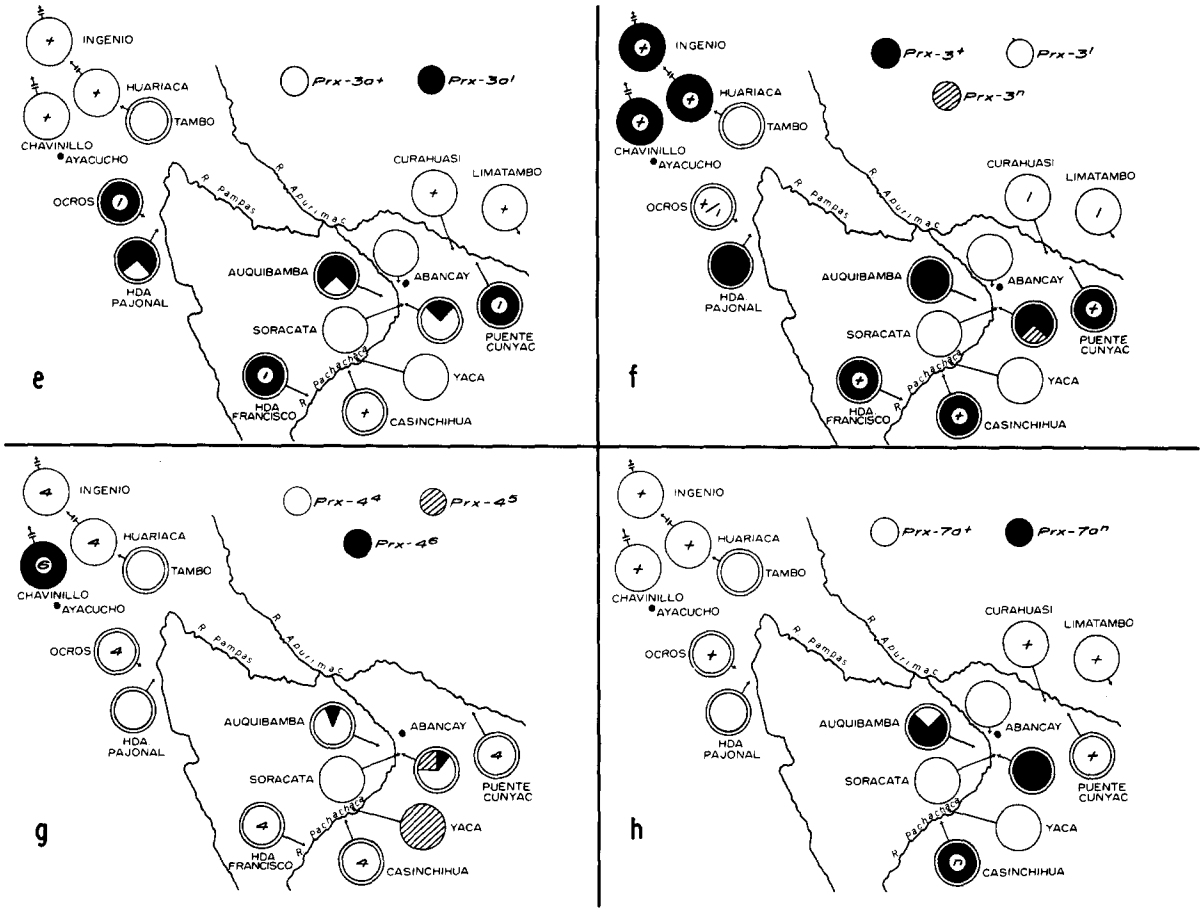
The geographic distribution of alleles for the polymorphic loci is shown in Fig.9, which illustrates the aforementioned degree of partial or complete genetic differentiation between the two species and comparative variation within each. It is of interest that the two species do not tend to resemble each other more in the sympatric area than in allopatric regions. As a matter of fact, for the differential loci, Prx-3 and Prx-7, the species are distinguished to a greater extent in the sympatric region. The contrast in variability between the two species in also exemplified by representative gels in Fig.8.

Discussion

As explained above, previous research established that members of the *minutum* complex are sufficiently isolated reproductively and are sufficiently distinguished morphologically from other *Lycopersicon* species to deserve specific status. Our research not only supports that thesis, but also reveals phylogenetic differentiation within the complex.

The weight of evidence in the foregoing presentation of facts and analysis reveals that differentiation between the two components - *L. chmielewskii* and *L. parviflorum* - has proceeded to the extent that they have reached specific status. This conclusion is based on considerations of comparative morphology, mating systems, ecological preferences, reproductive isolation, and sampling of allozymes.

The prime gross morphological difference is found in floral size and structure. The aggregate quantitative difference in floral apparatus - size of all parts of the inflorescence - amounts to a massive difference in the extent of display (Fig.3). Thus, at a distance, flowers of *L. chmielewskii* are conspicuous, whilst those of *L. parviflorum* can scarcely be de-



nate *L. chmielewskii;* single circles, *L. parviflorum.* Circles with allele symbols designate populations of single plants or those of indeterminate size (see text). Progeny tests of more than one plant are represented by circles without symbols. Further details in Tables 1 and 2

tected. Another consistent difference is strongly exserted stigmas of *L. chmielewskii* in contrast to stigmas slightly or not exserted in *L. parviflorum.* We suspect that these floral characteristics have evolved concomitantly with differentiation of the respective types of mating system in the two species. On one hand, floral showiness is an obvious device to attract pollinating insects and exserted stigmas, to facilitate cross-pollination in *L. chmielewskii.* On the other, non-exserted stigmas expedite self-pollination and the lack of selection pressure for attracting pollinating insects probably resulted in the evolution of depauperate flowers in *L. parviflorum.*

Differentiation is also manifest in the physiology of these species to the extent that they exhibit different ecological preferences. Such differences are apparent in the ecological niches that they occupy in nature and in the growing conditions that they tolerate in experimental cultures. Although these preferences are not strong enough to result in spatial isolation in nature, they are nevertheless so consistently observed that they must be real.

The compatibility tests show that it is possible to produce F_1 hybrids from both direct and reciprocal crosses and these hybrids are fully pollen fertile and produce seeds abundantly after selfing. The great difficulty experienced in germinating seeds produced by F_1 hybrids might constitute a barrier to gene exchange in nature.

Allozymes

The sampling of allozymes not only provides genetic information that is concordant with previously observed variation in morphological characters, but also greatly enhances our knowledge of the differentiation between the sibling species, the nature of their mating systems,

resemblance to other species, and even alludes to their evolution.

The data summarized in Tables 1 and 2 demonstrate a considerable hiatus between *L. chmielewskii* and *L. parviflorum:* at the Got-3 locus they possess no common alleles; the same holds for Prx-3 in the sympatric region; and at five other loci the majority of individuals of both are contrasted by their allozymes. In the latter group, *L. parviflorum* is nearly always homogeneous, whereas *L. chmielewskii* has two or more alleles. Thus, for half the tested loci, a major difference has been found between the two species.

The sibling species are also distinguished by the presence or absence of the defoliator (Df) gene. Chmielewski (1968) discovered this gene in several stocks of *L. parviflorum* and determined that it acts only in combinations with *esculentum* genes, not in pure species. According to our assays, however, it is absent in *L. chmielewskii,* which by virtue of this difference is more attractive to the plant breeder as a source of useful germ plasm.

In *L. parviflorum* only two of the tested enzymatic loci are polymorphic with a total of three variant alleles. In contrast, the bulk of the encountered polymorphism exists in *L. chmielewskii,* embracing all known *L. parviflorum* alleles and including 10 variant alleles. Every population of *L. chmielewskii* for which subsamples were collected varies for at least one locus. Furthermore, heterozygotes were detected for nearly every such locus. In all, none of the 21 progeny tests of *L. parviflorum* showed variability at any locus, whereas 15 of the 25 tests performed on *L. chmielewskii* did so. Small as this sampling is, the difference between the two sets of data when tested by contingency χ^2 is highly significant (16.1***, 1 df).

Differences in mating systems must account for at least part of this great difference in genetic variability. And the great differences in flower size, inflorescence size, and exposure of stigma suggest that *L. parviflorum* is certainly an intensive inbreeder, whilst *L. chmielewskii* would be expected to be subject to outcrossing. Although floral morphology is completely compatible with this explanation, we have not had opportunity to observe pollination biology in the wild to test this hypothesis.

Autogamy and the origin of *L. parviflorum*

The hypothesis for the evolution of these species that requires fewest assumptions and is most compatible with known facts is that *L. parviflorum* is the more recent and that it evolved from *L. chmielewskii.* Under certain unknown circumstances, selection favored the evolution of a highly self-pollinating form, which by acquiring this mating system, became reproductively isolated from the parental species. Strict autogamy is a mating system commonly exploited by aggressive, successful annual weeds (Stebbins 1957). The following evidence supports our contention that *L. parviflorum* evolved via autogamy: (1) the highly convincing evidence for the nature of the mating systems in the two species, (2) the remarkable uniformity of genotype in *L. parviflorum* throughout its range, despite sympatry and even intermingling with *L. chmielewskii,* and (3) *L. chmielewskii* embraces all alleles known in *L. parviflorum* except Got-3.

The aforementioned barrier to gene exchange engendered by autogamy in *L. parviflorum* is apparently reinforced by the inviability of propagules in the progeny of the interspecific hybrids. Such other factors as species constancy of pollinating insects, known for many closely related angiosperm species, might also exist to further strengthen the isolation.

Speciation via the simple device of autogamy is not uncommon amongst flowering plants. The present example is reminiscent of that in *Stephanomeria exigua* ssp. *coronaria* and *S. "malheurensis"* (Compositae-Cichorieae) (Gottlieb 1973), in which the evidence points to the sympatric origin via self-fertilization of the latter from the former. In both examples (1) the derivative selfer is vastly less variable than its putative parent, (2) all alleles of the former are known in the latter, (3) the two species are morphologically differentiated, (4) the obligately autogamous species has suffered reduction in corolla dimensions, and (5) the isolation is reinforced by various devices in addition to autogamy. They differ in the respect that only a single population of *S. "malheurensis"* is known, whilst *L. parviflorum* is very widely distributed. A similar example, although apparently not one of differentiation at the species level, is described by Brieger (1963) in *Encyclia odoratissima* (Orchidaceae-Epidendrinae) in southeastern Brazil. Var. *serroniana* is morphologically indistinguishable except for smaller flow-

ers with modifications to enforce self-pollination, and weaker plants. Its survival, despite weak growth, is attributed to its much greater rate of seed production. Its distribution is restricted to the central part of the territory occupied by the allogamous form. A somewhat similar example is cited by Brieger (loc.cit.) for *Epidendrum nocturnum* in the Guianas.

Relationships with other species

A brief consideration of the affiliations of *L. chmielewskii* and *L. parviflorum* with other tomato species is warranted. The compatibility relations clearly associate these sibling species with the *'esculentum* complex' (the taxa above the horizontal dividing line in Fig. 6) and reveal a strong and complex barrier with the *chilense-peruvianum* assemblage. Within the *'esculentum* complex', a higher degree of compatibility is exhibited with *L. esculentum, pimpinellifolium,* and *cheesmanii* - all with colored carotenoid pigments in their ripe fruits - and less compatibility with the green-fruited *L. hirsutum* and *S. pennellii*. Since the two sibling species are also green-fruited, compatibility reactions are not associated with fruit color, which was selected by Muller (1940) as the criterion for subgeneric classification. For morphological traits in general, the closest resemblance is with *L. pimpinellifolium.*

In respect to allozymes, we feel that the following brief comparisons are justified. Our surveys of the colored-fruited species have progressed sufficiently to permit comparisons, but we have thus far attained only a slight taste of the bewildering variability present in the other, outcrossing species. Extensive comparisons between all species would therefore be premature, but we can state that several bands appear to be unique in *L. chmielewskii* and *L. parviflorum*. We have observed the *parviflorum* Aps-2^n allele only in a tiny, isolated population of the Galapagan *L. cheesmanii*. The increased mobility of all four Got loci is not matched by anything we have yet seen in other species excepting Got-4 in *L. hirsutum, L. pimpinellifolium,* and *S. pennellii*. The advanced Prx-1^3 common to both sibling species resembles that of *pennellii,* although because less extreme, is probably a different allele. Such seemingly unique alleles argue for extensive differentiation of these species in isolation from the others - a conclusion that would not be difficult to reconcile with their present geographical isolation in the fastnesses of the interandean valleys.

Conclusion

Phylogenetic divergence between *L. chmielewskii* and *L. parviflorum* is manifest in their gross morphological, physiological, and allozymic phenotypes. Analysis of the data on genetic variability at all intraspecific levels and of floral characteristics leads to the conclusion that *L. parviflorum* is strictly autogamous whereas *L. chmielewskii* is the ancestral species from which *L. parviflorum* evolved by switching to autogamous reproduction. Strict inbreeding would permit this species to maintain its individuality even when cohabiting with *L. chmielewskii*. This reproductive isolation is apparently reinforced by low germination of intraspecific derivatives. We believe that the extent of differentiation between *L. chmielewskii* and *L. parviflorum* is sufficient to justify their classification as separate species.

Acknowledgements

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Literature

Barton, D.W.; Butler, L.; Jenkins, J.A.; Rick, C.M.; Young, P.A.: Rules for nomenclature in tomato genetics. J. Hered. 46, 22-26 (1955)

Berger, St.; Chmielewski, T.; Gronowska-Senger, A.: Studies on the inheritance of high ascorbic acid levels in tomatoes. Qual. Plant. Mat. Veg. 13, 214-218 (1966)

Brewer, G.J.: An Introduction to Isozyme Techniques. New York: Academic Press 1970

Brieger, F.G.: Evolução, filogenética, com referencia especial as plantas superiores. In: Pavan, C.; da Cunha, A.B.: Genetica. Comp. Edit. Nac., São Paulo, Brazil, 350-401 (1963)

Chmielewski, T.: Cytogenetical and taxonomical studies on a new tomato form. Part I. Genet. Polonica 3, 253-264 (1962)

Chmielewski, T.: A factor from *L. minutum* affecting the betacarotene content. Rept. Tomato Genet. Coop. 13, 10-11 (1963)

Chmielewski, T.: New dominant factor with recessive lethal effect in tomato. Genet. Polonica 9, 39-48 (1968a)

Chmielewski, T.: Cytogenetical and taxonomical studies on a new tomato form. Part II. Genet. Polonica 9, 97-124 (1968b)
Chmielewski, T.; Berger, St.: Inheritance of beta-carotene content in tomatoes. Genet. Polonica 3, 155-159 (1962)
Chmielewski, T.; Berger, St.: Genetic aspects of some carotenoids synthesis in tomatoes. Qual. Plant. Mat. Veg. 13, 219-227 (1966)
Chmielewski, T.; Gronowska, A.; Prończuk, A.: Studies on the character of increased concentration of vitamin C in tomato fruit obtained from the wild *Lycopersicon minutum* species. Genet. Polonica 5, 371-375 (1964)
Chmielewski, T.; Rick, C.M.: *Lycopersicon minutum*. Rept. Tomato Genet. Coop. 12, 21-22 (1962)
Clayberg, C.D.; Butler, L.; Rick, C.M.; Young, P.A.: Second list of known genes in the tomato. J. Hered. 51, 167-174 (1960)
Clayberg, C.D.; Butler, L.; Kerr, E.A.; Rick, C.M.; Robinson, R.W.: Third list of known genes in the tomato. J. Hered. 57, 189-196 (1966)
Gottlieb, L.D.: Genetic differentiation, sympatric speciation, and the origin of a diploid species of *Stephanomeria*. Amer. J. Botany 60, 545-553 (1973)
Muller, C.H.: A revision of the genus *Lycopersicon*. U.S.D.A. Misc. Publ. 328, 29 pp. (1940)
Rick, C.M.: Potential genetic resources in tomato species: clues from observations in native habitats. In: Srb, A.M., Genes, Enzymes, and Populations, 255-269. New York: Plenum Press 1973
Rick, C.M.: High soluble-solids content in large-fruited tomato lines derived from a wild green-fruited species. Hilgardia 42, 493-510 (1974)
Rick, C.M.; Fobes, J.F.: Allozymes of Galápagos tomatoes: polymorphism, geographic distribution, and affinities. Evolution (in press, 1975)
Rick, C.M.; Zobel, R.W.; Fobes, J.F.: Four peroxidase loci in red-fruited tomato species: genetics and geographic distribution. Proc. nat. Acad. Sci. USA 71, 835-839 (1974)
Scandalios, J.G.: Genetic control of multiple molecular forms of enzymes in plants: a review. Biochem. Genetics 3, 37-79 (1969)
Shaw, C.R.; Prasad, R.: Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genetics 4, 297-320 (1970)
Stebbins, G.L.: Self-fertilization and population variability in the higher plants. Amer. Natural. 91, 337-354 (1957)

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