

RELATIONSHIPS, ORIGIN, AND DIVERSITY OF GALÁPAGOS TOMATOES: IMPLICATIONS FOR THE CONSERVATION OF NATURAL POPULATIONS^{1,2}

FERNANDO NUEZ,³ JAUME PROHENS, AND JOSÉ M. BLANCA

Centro de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain

Endemic Galápagos tomatoes (*Lycopersicon cheesmanii*) are of great value for cultivated tomato (*L. esculentum*) breeding, and therefore their conservation is of significance. Although within *L. cheesmanii* there is heterogeneity for many traits and formal infra-specific classification is not justified, here we distinguish three forms, without taxonomic significance, of *L. cheesmanii* that are of interest to breeders because of their distinctive morphology and habitat preferences: *L. cheesmanii* 'short' (one- to two-pinnate leaves, short internodes, and coastal habitats), *L. cheesmanii* 'long' (one- to two-pinnate leaves, long internodes, and inland habitats), and *L. cheesmanii* forma *minor* (three- to four-pinnate leaves, short internodes, and coastal habitats). In a recent survey of tomato populations in the Galápagos Islands, we found that several populations of *L. cheesmanii* reported 30–50 years earlier had disappeared, mostly as a consequence of human activity. In addition, a previously unreported invasive wild red-fruited form, which we named *L. esculentum* 'Gal cer,' was found on the island of Santa Cruz. The total diversity (estimated with amplified fragment length polymorphisms [AFLPs]) within *L. cheesmanii* ($H_T = 0.051$) is almost as high as that for the mainland wild species *L. pimpinellifolium* ($H_T = 0.072$). *Lycopersicon esculentum* 'Gal cer,' on the other hand, has a much lower diversity ($H_T = 0.014$). Comparison of AFLP fragments shared by *L. esculentum* 'Gal cer' with other species showed that it is closely related to weedy tomato *L. esculentum* var. *cerasiforme* and, therefore, likely of recent origin. Genetic differentiation among the three native *L. cheesmanii* forms is low ($G_{ST} = 0.235$), indicating that they share a common genetic background. Nonetheless, *L. cheesmanii* 'short' is about twice as diverse as *L. cheesmanii* 'long' or *L. cheesmanii* f. *minor*. UPGMA cluster and principal components analysis distinguish four groups within *Eulycopersicon*: *L. pimpinellifolium*, cultivated *L. esculentum*, *L. esculentum* var. *cerasiforme* including *L. esculentum* 'Gal cer,' and *L. cheesmanii*. The geographic distance and genetic distance in the wild forms of Galápagos tomatoes were not correlated. Apart from the pressure of humans, some native *L. cheesmanii* populations, especially *L. cheesmanii* 'long,' might be displaced by invasive *L. esculentum* 'Gal cer' because they share a similar habitat. We did not find evidence of intercrossing of *L. cheesmanii* with introduced *L. esculentum*, but occasional hybridization that contributes to loss of genetic integrity of *L. cheesmanii* cannot be ruled out. Establishment of reserves of *L. cheesmanii* to protect this species from introduced herbivorous animals and from hybridization with *L. esculentum* 'Gal cer' would help to conserve *L. cheesmanii*. Furthermore, accessions collected by C. M. Rick and others in the 1950s–1970s and now stored in germplasm banks could be used to reinstate some extinct populations.

Key words: amplified fragment length polymorphisms (AFLPs); endangered species; Galápagos Islands; genetic resources; *Lycopersicon cheesmanii*; *Lycopersicon esculentum*; Solanaceae; tomato.

Tomato (*Lycopersicon esculentum* Mill.) is the world's most important vegetable crop in economic terms. Wild relatives of tomato have great value as genetic resources (Stevens and Rick, 1986; Nuez, 1995). Consequently, conservation of wild species of *Lycopersicon*, not only ex situ in germplasm banks, but also in situ in the form of natural populations that conserve the genes in their place of origin, is a high priority.

Multiple molecular data sets indicate that tomatoes are deeply nested with *Solanum* and because of this, Spooner et al. (1993) transferred them to *Solanum* sect. *Lycopersicon* and provided new combinations for many of the *Lycopersicon* species. However sound the phylogenetic interpretation, the taxonomic treatment of tomatoes within the genus *Solanum* is debatable, and both options are open (Lester, 1991; Spooner

et al., 1993). The argument that because tomatoes have evolved from within *Solanum* and thus must be included in *Solanum* to produce a monophyletic phylogenetic classification and avoid paraphyly is subject to discussion; a Linnaean classification without paraphyletic taxa is a logical impossibility (Brummitt, 2002; R. N. Lester, University of Birmingham, personal communication). For this reason, for the purpose of clarity, and because numerous historical and present references are cited within this report, we accept the paraphyly and use the original *Lycopersicon* names.

The genus *Lycopersicon* is divided into two subgenera, *Eulycopersicon* and *Eriopersicon* (Muller, 1940). The former includes the red-, orange-, or yellow-fruited species *L. esculentum*, *L. cheesmanii* Riley, and *L. pimpinellifolium* (Jusl.) Mill. *Eriopersicon* comprises the green-fruited species *L. chilense* Dun., *L. chmielewskii* Rick, Kesicki, Fobes & Holle, *L. hirsutum* Humb. & Bonpl., *L. parviflorum* Rick, Kesicki, Fobes & Holle, *L. pennellii* (Corr.) D'Arcy, and *L. peruvianum* (L.) Mill. (Rick, 1979; Taylor, 1986; Warnock, 1988). Although the cultivated tomato was probably domesticated in Mexico (Jenkins, 1948), all wild species are naturally distributed in mainland South America (Warnock, 1991; Esquinas-Alcázar and Nuez, 1995), except for *L. cheesmanii*, which is endemic to the Galápagos Islands (Rick, 1956). Like most of the Galá-

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² This paper is dedicated to the memory of Dr. Charles M. Rick, who made the greatest contributions to the biosystematics of *Lycopersicon*.

³ E-mail: fnuez@btc.upv.es.

pagos flora and fauna, *L. cheesmanii* has evolved in isolation from mainland species and has acquired a combination of morphological and physiological characteristics unique in the genus, such as orange-red or orange-yellow fruits, yellow-green foliage, minute seed size, and exceptional seed dormancy (Mackinney et al., 1954; Rick, 1956, 1963).

Lycopersicon cheesmanii is of great interest for tomato breeding: it is easily crossed with the cultivated tomato to produce fertile offspring, and it is a source of variation for several traits of agronomic interest (Rick, 1979; Nuez, 1995). Some forms of *L. cheesmanii* that thrive near the coastline in extremely arid and saline environments are sources of tolerance to salinity and to drought (Rush and Epstein, 1981). *Lycopersicon cheesmanii* is also of great interest in breeding for quality as it has high sugar (Hewitt and Garvey, 1987; Poysa, 1993) and β -carotene contents (Mackinney et al., 1954; Stommel, 2001). The jointless pedicel of the fruit of some accessions, a trait conferred by the gene *j-2*, has also been used in breeding processing tomatoes as it facilitates separation from the calyx during mechanical harvest (Rick, 1967). Other *L. cheesmanii* accessions are resistant or tolerant to pathogens such as *Alternaria alternata* (Cassol and St. Clair, 1994) or tomato yellow leaf curl virus (Hassan et al., 1984; Picó et al., 1996). Unfortunately, introduced plants and animals, particularly feral donkeys and goats, and human activity threaten *L. cheesmanii* populations (Rick and Bowman, 1961; Jackson, 1994). Therefore, conservation of *L. cheesmanii* is urgently needed.

Within *L. cheesmanii*, one well-defined infraspecific form (*L. cheesmanii* forma *minor*), characterized by having a distinctive syndrome of morphological traits, can be distinguished (Rick, 1983). Plants of *L. cheesmanii* f. *minor* can be easily identified by their three- to four-pinnately compound leaves. Rick (1980) demonstrated that this trait is under the control of a single gene (*Pts*) with incomplete dominance. In the remainder of the species (one- to two-pinnately compound leaves), which is considered as the typical or straight *L. cheesmanii*, the assortment of many traits of taxonomic interest is too heterogeneous to permit classification into subgroups (Rick, 1983). However, two different forms without formal taxonomic designation can be distinguished; they vary greatly for a trait (internode length) that has a large impact on the plant morphology. The two forms also have different habitat preferences (coastal or inland). One, which we have labeled *L. cheesmanii* 'short,' corresponds to forms with short internodes (2–4 cm) and usually thrives near coastal areas. The other, which we have called *L. cheesmanii* 'long,' has longer internodes (5–8 cm) and usually grows inland. Distinction between these two forms, despite not corresponding to different formal taxa, may be of interest to breeders. In addition to these endemic forms, typical cultivated tomato (*L. esculentum*) as well as weedy cherry tomato (*L. esculentum* var. *cerasiforme* (Dun.) Gray), have also been reported from the islands (Rick, 1956). Recently, we also found wild and weedy populations of plants that bear small (<15 mm) red fruits and seem to be derivatives of the introduced *L. esculentum* var. *cerasiforme*. We have given them the provisional name *L. esculentum* 'Gal cer' to distinguish them from the typical feral forms of tomato *L. esculentum* var. *cerasiforme*, which have larger fruits (>15 mm).

Studies on the molecular diversity of Galápagos tomatoes and their implications for conservation have been limited. The only comprehensive work was performed by Rick and Fobes

(1975) with allozymes. They found that diversity among *L. cheesmanii* accessions was high, but that within-accession allozyme variation in *L. cheesmanii* was very low and comparable to the degree of genetic uniformity characteristic of pure lines of domesticated *L. esculentum*. This is expected as *L. cheesmanii* stigmas are inserted or only slightly exerted, which leads to autogamy, and because of the consequent rapid fixation of alleles and differentiation between populations (Rick, 1983). Since the valuable contributions of Rick and co-workers (Rick, 1956, 1963, 1971; Rick and Bowman, 1961; Rick and Fobes, 1975) on the biosystematics, diversity, relationships with the continental species, and evolution of Galápagos tomatoes, there have been no further studies on these subjects for *L. cheesmanii*. Although *L. cheesmanii* has been included in many studies on the molecular diversity of the genus *Lycopersicon*, in general, only one or a few accessions have been studied at a time (Palmer and Zamir, 1982; Miller and Tanksley, 1990; Bretó et al., 1993; Williams and St. Clair, 1993; Álvarez et al., 2001; Marshall et al., 2001; Peralta and Spooner, 2001; Nesbitt and Tanksley, 2002).

The optimal use of genetic resources in present and future breeding requires implementing strategies to conserve wild germplasm. Because of the unique characteristics of Galápagos tomatoes, their utility for tomato breeding, and their susceptibility to genetic erosion, it is necessary to have updated studies on their characteristics, distribution, relationships, origin, and diversity. Amplified fragment length polymorphisms (AFLPs) give a high level of polymorphism in *Solanaceae* (Milbourne et al., 1997; Kardolus et al., 1998), allow a large number of loci to be scored in a single reaction, and have a much better repeatability among laboratories than other markers such as random amplified polymorphisms of DNA (RAPDs) (Jones et al., 1997). Therefore, AFLPs may be useful to address these issues in Galápagos tomatoes.

MATERIALS AND METHODS

Collection of *Lycopersicon* populations from the Galápagos Islands—Two of us (F. Nuez and J. Prohens) took part in a *Lycopersicon*-collecting expedition in 2000 in the central islands of the Galápagos (Baltra, Bartolomé, Champion, Corona del Diablo, Daphne Mayor, Daphne Menor, Edén, Enderby, Plazas, Pinzón, Rábida, Santa Cruz, Santa Fé, Santa María Santiago, Seymour Norte, Sin Nombre, Sombrero Chino, and Sucre) (Fig. 1). Islands were circumnavigated close to the coast in a small boat to search for *Lycopersicon* plants near the shore. Wherever *Lycopersicon* plants were detected, we landed and searched for more plants or remains of them in the area, both near the shore and inland (up to several hundred meters when possible). In addition, we landed and made exhaustive searches in the areas where *L. cheesmanii* populations had been reported previously (Rick, 1956, 1963, 1971, 1983; Rick and Fobes, 1975; C. M. Rick Tomato Genetics Resource Center [TGRC] passport data website: <http://tgrc.ucdavis.edu>). Apart from the search for coastal populations, we also prospected inland on Santa Cruz Island.

Passport data for each accession were taken as recommended by the International Plant Genetic Resources Institute (IPGRI, 1996). Longitude and latitude of each population were determined using a global positioning system (GPS; Magellan Meridian XL, San Dimas, California). Altitude was measured either with the GPS or with a barometric altimeter.

Plant materials used in the analysis of variation—The plant materials used to study both morphological and molecular variation consisted of 43 accessions. Of these, 15 were wild accessions we collected in the Galápagos, selected to represent the whole range of different morphological forms found (*L. cheesmanii* 'short,' *L. cheesmanii* 'long,' *L. cheesmanii* f. *minor*, and *L. esculentum* 'Gal cer') and the different collection sites; eight were *L. chees-*

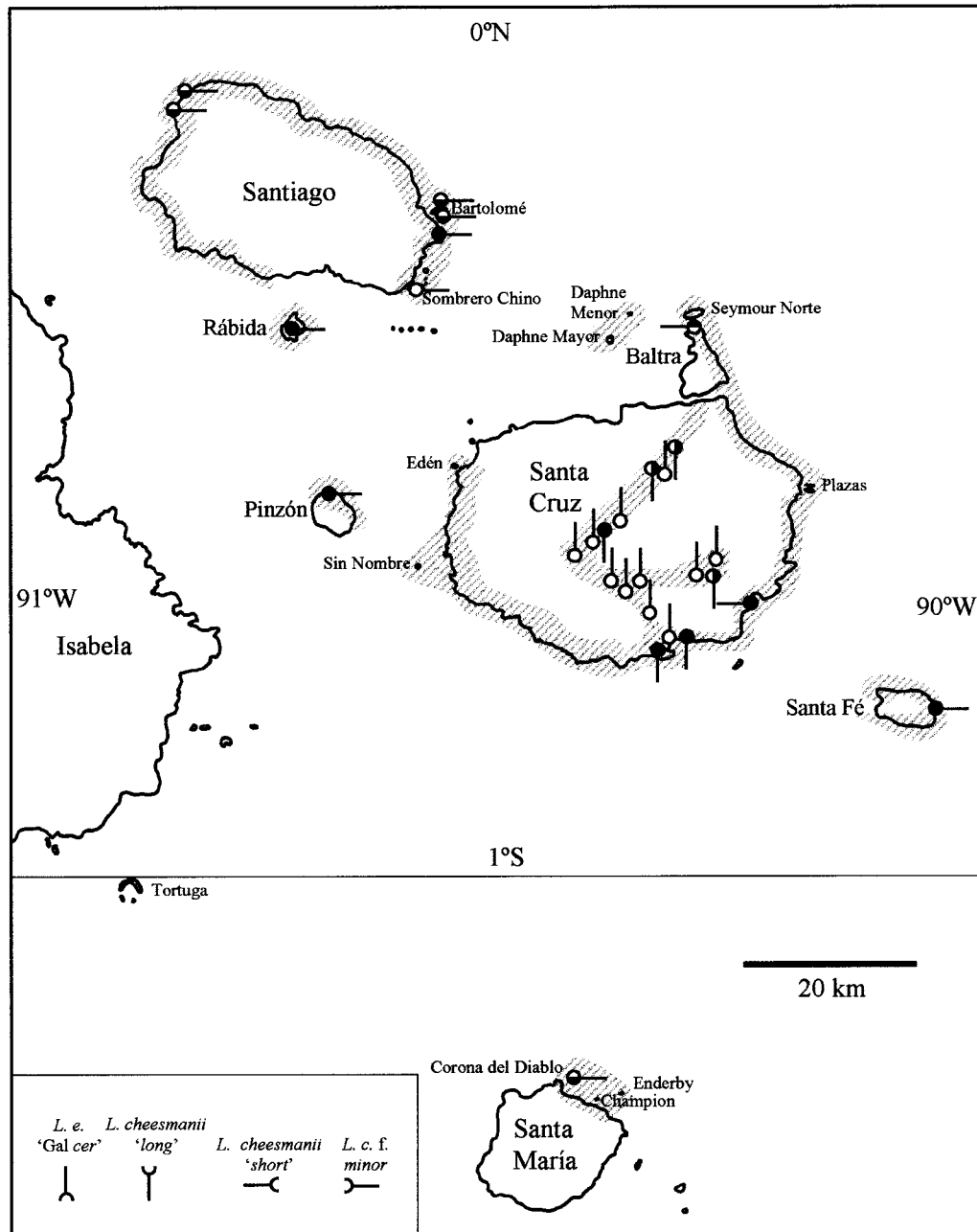


Fig. 1. Geographic distribution of wild *Lycopersicon* forms in the areas surveyed (shaded) in the central Galápagos Islands. The different forms are indicated by ideograms, as represented in the lower left corner. Open circles represent populations that we found and were not reported previously (Rick, 1956, 1963, 1971; Rick and Bowman, 1961; Rick and Fobes 1975; TGRC passport data); solid circles represent populations previously reported but for which we have found neither plants nor remains of plants; half-solid circles represent populations reported previously and also found by us.

manii accessions from the TGRC, representative of the different forms stored in this gene bank (*L. cheesmanii* and *L. cheesmanii* f. *minor*); three were accessions of weedy *L. esculentum* var. *cerasiforme* collected in the Galápagos; 11 were accessions from the continental *L. pimpinellifolium*, the wild species genetically closest to *L. cheesmanii* (Rick and Fobes, 1975; Esquinas-Alcázar and Nuez, 1995), and one accession of cultivated *L. esculentum*, and of each of the wild subgenus *Eriopersicon* species: *L. chilense*, *L. hirsutum*, *L. parviflorum*, *L. pennellii*, and *L. peruvianum* (Table 1). Pairwise geographic distances of selected accessions were calculated either from the GPS position, if available, or from the approximate position of the collection site.

Lycopersicon pimpinellifolium was included in the study to compare the

diversity of this wild mainland species with *L. cheesmanii* and to study its relationships with the small-fruited *L. esculentum* 'Gal cer.' All TGRC (LA codes) accessions (12 total) except two (LA1401 and LA2857), agreed with the taxonomic classification of TGRC. LA1401 is classified by TGRC as *L. cheesmanii* f. *minor*. However, on the basis of leaf subdivision (leaflets not subdivided) and other traits (such as low hairiness) that do not characterize *L. cheesmanii* f. *minor*, we treated it as a "typical" *L. cheesmanii*. On the other hand, LA2857 is classified by the TGRC as *L. pimpinellifolium*. However, in our characterization, trial fruits had a diameter >20 mm and other characteristics (such as a serrate leaf margin and seeds longer than 1.5 mm) typical of *L. esculentum* var. *cerasiforme*. We attribute these differences in

the classification to an environmental effect and/or other uncontrolled circumstances, not to inaccuracies in the TGRC passport data. For our analyses we treated these accessions as identified by us.

Growing conditions—Seeds were subjected to thermotherapy (24 h at 80°C) to avoid infection by seed-transmitted pathogens. Seeds were germinated on moistened filter paper in petri dishes. Seed testa of some Galápagos accessions were excised to ensure successful germination (Rick, 1956; Rick and Bowman, 1961).

Germinated seeds were transferred to seedling trays and were kept in a growth chamber until their transplantation to a glasshouse. Plants were spaced at 0.3 m within the row and 1 m between rows, trained with vertical strings, and watered by drip irrigation.

To improve fruit set, inflorescences were shaken three times a week with a mechanical vibrator to favor the release of pollen from anthers. For each of the accessions of the self-incompatible species *L. chilense*, *L. hirsutum*, *L. pennellii*, and *L. peruvianum*, hand pollinations using a mixture of pollen of several plants from the same accession were performed to ensure some fruit set.

Morphological characterization—Six plants per accession were characterized using the International Plant Genetic Resources Institute (IPGRI) descriptors list for tomato (IPGRI, 1996). Characters evaluated included qualitative and quantitative traits of the plants, flowers, inflorescences, and fruit. Mean values for each accession were obtained as the mean of six plants, for which a variable number of flowers/fruits per plant was evaluated.

DNA extraction and AFLP analysis—For each accession, genomic DNA was isolated from a mixture of young leaves from six plants using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the protocol of the manufacturer. DNA concentration was quantified on agarose, and a 0.25- μ g DNA sample was digested by the enzyme combination *EcoRI* and *MseI* at 37°C for 2 h. Ligation was performed with the AFLP Core Reagent Kit (Invitrogen, Carlsbad, California, USA) following the instructions of the manufacturer. After ligation, the reaction mixture was diluted 1 : 10 in Tris-EDTA (TE) buffer.

For the preselective amplification, a 5- μ L aliquot from the DNA dilution was added to a 25- μ L solution containing 2.5 μ L of 10 \times buffer, 0.5 μ L of primer *EcoA* (10 μ mol/L), 0.5 μ L of primer *MseC* (10 μ mol/L), 1.0 μ L of dNTPs (10 mmol/L), and 0.8 units of *Taq* polymerase (Roche, Basel, Switzerland). After preamplification, DNA was diluted again 1 : 10 in TE buffer. The selective amplification was performed on 2- μ L aliquots using four combinations of primers (Table 2). DNA fragments were separated in an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, California, USA). Resulting fragments were scored as binary traits (1 = present, 0 = absent) using Genographer 1.6 software (Montana State University, Bozeman, Montana, USA).

Cluster analysis and estimates of genetic distances—Pairwise genetic similarities were estimated with the Dice (Sorensen) similarity coefficient $S_{ij} = 2a/(2a + b + c)$ and with the Jaccard coefficient $J_{ij} = a/(a + b + c)$, where a is the number of bands shared by i and j , b is the number of bands present in i and absent in j , and c is the number of bands present in j and absent in i . Correlation between similarity matrices was assayed with a Mantel (1967) test.

The resulting genetic similarity matrices were used to generate unweighted pair group method using arithmetic means (UPGMA) phenograms using the NTSYSpc2.0 software package (Rohlf, 1996). The reliability and robustness of the phenograms were tested by bootstrap analysis with 1000 replications to assess branch support. A limit of 50% was used to indicate statistical support for the topology at a node (Highton, 1993). A principal coordinate analysis (PCA) based on genetic similarity matrices was performed using the DCENTER and EIGEN algorithms of the NTSYSpc2.0 software package.

Genetic diversity was estimated with the proportion of polymorphic fragments (P_p) and the total diversity (H_T) (Nei, 1973). Total diversity was partitioned into diversity among (D_{ST}) and within (H_S) groups. The relative mag-

nitude of gene differentiation among groups (G_{ST}) was calculated as the ratio D_{ST}/H_S (Nei, 1973). The estimates of genetic identity (J) and standard genetic distance (D) were calculated as indicated in Nei (1974).

RESULTS

Taxa and forms of *Lycopersicon* from the Galápagos Islands—*Lycopersicon cheesmanii* accessions display a heterogeneous assortment for several traits among accessions that does not support a classification of *L. cheesmanii* into subgroups with taxonomic consideration (Rick, 1983). However, information collected during the expedition, together with subsequent observations of plants in cultivation and previously published information on the subject, allowed us to distinguish three forms of *L. cheesmanii* with different gross morphology (Fig. 2) and/or ecological preferences. Because these differences among the forms of *L. cheesmanii* might affect their adaptation and genetic differentiation, we have studied them separately. This separation will give more information of relevance regarding the infraspecific variation, diversity, and conservation strategies than if all *L. cheesmanii* accessions were forced into a single group. Similarly, the newly reported *L. esculentum* 'Gal cer' has been studied as a separate entity of *L. esculentum* var. *cerasiforme* to provide more information on its origin and diversity. The main characteristics and differences between these wild forms are the following:

1. *L. cheesmanii* 'short' (Fig. 2a)—This type form was found only as coastal populations on cliffs and in open, undisturbed areas. Germination is slow, and in many cases, the seed testa must be excised to allow the radicle to emerge. The plants have pale green foliage and short internodes (2–4 cm), characteristics that persist even in good soil and uniform conditions. Leaves are pinnately compound with first- and second- order pinnate subdivisions. Leaves and stems are very brittle. Under our greenhouse conditions (40° N, Valencia, Spain), they do not flower from mid-November to mid-February. However, it is not known if this is due to the fact that this form requires a light regime typical of its region of origin (12 h light : 12 h dark) or that it flowers better in bright conditions of Spain's spring/summer. Ripe fruit color is orange-red.
2. *L. cheesmanii* 'long' (Fig. 2b)—This is usually found inland. Its foliage is darker than that of *L. cheesmanii* 'short' and has longer internodes (5–8 cm), similar to those of the mainland *Eulycopersicon* species. It germinates rapidly. Leaflets have small lobes. Leaves and stems are not particularly brittle. In our greenhouse conditions, it has flowering requirements similar to *L. cheesmanii* 'short.' Ripe fruit color varies from pale straw-yellow to dull orange-yellow.
3. *L. cheesmanii* f. *minor* (Fig. 2c)—Like *L. cheesmanii* 'short,' it is usually found in undisturbed coastal areas, has strong seed dormancy, has foliage that is pale green, and its internodes are short (2–4 cm). However, the morphology of the plant is very distinctive: leaves are three- to four-pinnate, leaflets are deeply lobed, all parts of plant are excessively hairy, stems are thick, and leaves erect. Flowering requirements are similar to *L. cheesmanii* 'short' and *L. cheesmanii* 'long.' Ripe fruit color is similar to the 'short' form.
4. *L. esculentum* 'Gal cer' (Fig. 2d)—This truly red-fruited form has not been reported previously in the Galápagos

TABLE 1. List of accessions used for the study of morphological and molecular variation. Germplasm samples labeled LA are deposited in TGRC (Davis, California, USA), while the rest of the accessions are conserved in a seed bank at COMAV (Valencia, Spain).

Accession	Island or country	Sampling sites	Latitude	Longitude	Altitude (m)	Year of collection
Subgenus <i>Edycopersicon</i>						
<i>Lycopersicon cheesmanii</i> 'short'						
GLP-57	Balra	Canal Norte	0°24'45"S	90°17'04"W	5	2000
GLP-65	Balra	Canal Norte	0°24'52"S	90°17'24"W	5-10	2000
LA1401	Isabela	North of Punta Tortuga	—	—	6	1971
LA3124	Santa Fé	Beach trail	—	—	1	1991
<i>Lycopersicon cheesmanii</i> 'long'						
GLP-27	Santa Cruz	Rd. Channel-Sta. Rosa (17 km from Channel)	0°33'37"S	90°19'58"W	330	2000
LA0521	Femandina	Shore of interior lake	—	—	600	1957
LA1449	Santa Cruz	Barranco near seismograph	—	—	20-30	1971
<i>Lycopersicon cheesmanii</i> f. <i>minor</i>						
GLP-34	Santiago	Caleta Bucanero	0°09'33"S	90°49'24"W	10	2000
GLP-35	Bartolomé	Sudwest	0°17'24"S	90°33'32"W	10	2000
GLP-39	Bartolomé	North	0°16'54"S	90°33'07"W	5	2000
GLP-44	Bartolomé	West	0°17'09"S	90°32'30"W	5	2000
GLP-54	Sombbrero Chino	North	0°22'01"S	90°35'05"W	15	2000
LA0317	Bartolomé	—	—	—	15	1954
LA0483	Femandina	Inside crater	—	—	—	1956
LA0532	Pinzón	Northwest	—	—	5	1958
LA1508	Corona del Diablo	Inner wall of crater	—	—	5	1972
<i>Lycopersicon esculentum</i> 'Gal cer'						
GLP-12	Santa Cruz	Rd. Pto. Ayora-Balra (near the Volcanes Gemelos)	0°38'12"S	90°23'51"W	500	2000
GLP-14	Santa Cruz	El Cascajo	0°40'10"S	90°15'55"W	300	2000
GLP-17	Santa Cruz	Barranco west of Puerto Ayora	0°44'49"S	90°19'10"W	10	2000
GLP-18	Santa Cruz	Puerto Ayora	0°44'49"S	90°18'53"W	5	2000
GLP-20	Santa Cruz	Rd. Camote a Cascajo	0°39'32"S	90°16'48"W	450	2000
GLP-24	Santa Cruz	Rd. Bellavista-Cascajo	0°31'40"S	90°19'21"W	360	2000
GLP-30	Santa Cruz	Rd. Channel-Sta. Rosa (18 km from Channel)	0°33'41"S	90°20'01"W	360	2000
<i>Lycopersicon esculentum</i> var. <i>cerasiforme</i>						
GLP-26	Santa Cruz	Rd. Bellavista-Cascajo	0°31'40"S	90°19'21"W	380	2000
LA2857	Isabela	Villamil	—	—	—	—
LA3123	Santa Cruz	Rd. Puerto Ayora-Balra	—	—	—	1991
<i>Lycopersicon esculentum</i>						
NE-1	Spain	Breeding line developed by F. Nuez	—	—	—	—
<i>Lycopersicon pimpinellifolium</i>						
ECU-417	Ecuador	Sarayunga (Rd. Santa Isabel)	3°19'08"S	79°35'28"W	575	1996
ECU-440	Ecuador	Chaur Pamba (Azuay)	3°18'49"S	79°20'88"W	1453	1996
ECU-443	Ecuador	Cañón Santa Isabel	3°18'56"S	79°21'14"W	1345	1996
ECU-591	Peru	Department of Lambayeque (Rd. to Olmos)	5°49'05"S	79°49'59"W	150	1998
ECU-597	Peru	Rd. Olmos-Jaén (diversity hotspot)	5°59'42"S	79°43'03"W	230	1998
ECU-604	Peru	Rd. Piura-Talara	4°51'01"S	80°50'57"W	25	1998
ECU-655	Peru	Rd. Olmos-Jaén (diversity hotspot)	5°59'42"S	79°43'03"W	230	1998
ECU-660	Peru	San Isidro	4°48'37"S	78°17'46"W	200	1998
ECU-927	Ecuador	Rd. Macará-Loja	4°08'37"S	79°50'50"W	800	1999
ECU-951	Ecuador	78 km from Cuenca in direction to Machala	3°18'19"S	79°21'07"W	1580	1999
PE-14	Peru	Casma Valley	—	—	—	1983

TABLE 1. Continued.

Accession	Island or country	Sampling sites	Latitude	Longitude	Altitude (m)	Year of collection
Subgenus <i>Eriopersicon</i>						
<i>Lycopersicon chilense</i>						
LA1960	Peru	Osmore River	—	—	1950	1979
<i>Lycopersicon hirsutum</i>						
E-302	Ecuador	Parroquia Taquil (Loja)	3°53'50"S	79°17'42"W	2000	1996
<i>Lycopersicon parviflorum</i>						
LA0247	Peru	Chavinillo (Huánuco)	—	—	—	1951
<i>Lycopersicon pennellii</i>						
PE-45	Peru	Km 58 Rd. Santa-Huaraz (Ancash)	—	—	—	1983
<i>Lycopersicon peruvianum</i>						
PE-16	Peru	San José (Cajabamba)	—	—	—	1983

TABLE 2. Oligonucleotide adaptors and primers used for the AFLP analysis.

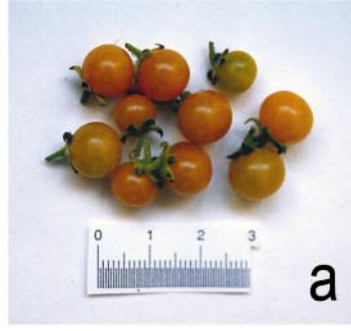
	Restriction enzyme	Sequence
E-0 Adaptor	EcoRI	5'-CTCGTAGACTGCGTACC-3' 3'-CTGACGCATGGTTAA-5'
M-0 Adaptor	MseI	5'-GACGATGAGTCCTGAG-3' 3'-TACTCAGGACTCAT-5'
E-A	EcoRI	5'-AGACTGCGTACCAATTCA-3'
M-C	MseI	5'-GATGAGTCCTGAGTAAC-3'
E-ACA	EcoRI	5'-AGACTGCGTACCAATTCACA-3'
E-AAC	EcoRI	5'-AGACTGCGTACCAATTCAAC-3'
M-CAA	MseI	5'-GATGAGTCCTGAGTAACAA-3'
M-CTA	MseI	5'-GATGAGTCCTGAGTAAC-3'

(Rick, 1956, 1963, 1971; Rick and Bowman, 1961; Rick and Fobes, 1975). Morphologically, these plants are very similar to *L. esculentum* var. *cerasiforme*, but fruits are much smaller (always <15 mm). They are found inland, in areas of high soil humidity in highly disturbed areas (road and trail margins, works areas, dumps, gardens). Like domesticated *L. esculentum* and feral *L. esculentum* var. *cerasiforme*, they do not need long (or bright) days to flower. The ripe fruit is deep red and is attractive to the Galápagos mockingbird (*Nesomimus parvulus*), which we observed carrying ripe fruits in their beaks.

Apart from these four wild forms, on the inhabited islands we also found cultivated (*L. esculentum*) and feral (*L. esculentum* var. *cerasiforme*) tomato. The feral tomato is found as a weed in vegetable gardens and trail margins. Galápagos accessions present the typical traits of this taxon (Fig. 2e).

There are important differences among *L. cheesmanii*, *L. esculentum*, and the wild continental species *L. pimpinellifolium* in the quantitative morphological traits measured in this work (Table 3). Internode length is much shorter in *L. cheesmanii* 'short' and *L. cheesmanii* f. *minor* than in the other taxa. Although there are not absolute differences in the size of the flower among the taxa, *L. cheesmanii* has a lower petal length to sepal length ratio than *L. esculentum* 'Gal cer' or *L. pimpinellifolium* (Table 3). Another remarkable feature of *L. cheesmanii* is that stigmas are only slightly exerted (always less than 0.4 mm), much less than stigmas of *L. esculentum* or *L. pimpinellifolium* (Table 3). There is also an important difference between *L. cheesmanii* and the other *Eulycopersicon* taxa in the flowering requirements. Although in their native environment and in other places at lower latitudes than Valencia, such as Davis, California (R. Chetelat, TGRC, personal communication), *L. cheesmanii* flowers all the year round if the conditions are favorable for plant growth and development, in the latitudes of Spain (40° N) they do not flower from mid-November to mid-February.

Regarding fruit traits, the fruits of *L. cheesmanii* 'short' and *L. cheesmanii* f. *minor* are generally smaller than for the accessions of the other taxa studied here, not reaching 10 mm in length or breadth; the accessions of *L. cheesmanii* 'long,' *L. esculentum* 'Gal cer,' and *L. pimpinellifolium* studied here have fruit lengths and breadths that vary from 11 to 13 mm; Galápagos *L. esculentum* var. *cerasiforme* fruits are much larger, with a breadth greater than 20 mm. Differences in soluble solids content (SSC) are also noteworthy; the highest values were those of the coastal forms of *L. cheesmanii*. In particular, fruit of *L. cheesmanii* f. *minor* have an average SSC that ex-



ceeds 14°Brix, while those of *L. cheesmanii* 'short' average 10°Brix, much higher than the common values of the other accessions studied here belonging to other *Eulycopersicon* taxa and forms. *Lycopersicon esculentum* 'Gal cer' has an SSC similar to that found in the Galápagos accessions of *L. esculentum* var. *cerasiforme*, which is lower than that of the mainland accessions of *L. pimpinellifolium* (Table 3).

Survey of *Lycopersicon* populations in the Galápagos Islands—A total of 37 populations of *Lycopersicon* were found in distinct geographical locations in our field work in 2000 (Fig. 1). Of these, 12 were coastal populations, with three of these belonging to *L. cheesmanii* 'short' and nine to *L. cheesmanii* f. *minor* found in environments undisturbed by humans. All of the *L. cheesmanii* 'short' populations that we found were situated in the same geographical area (northern channel of Baltra Island). *Lycopersicon cheesmanii* f. *minor* was more widespread. The largest coastal populations were found on the small islands of Bartolomé and Sombrero Chino, where there are no introduced herbivorous animals. On these islands, the plants were found in lava flow crevices, and in general, populations consisted of many plants (>50), most young and vigorous. On the tiny island of Corona del Diablo (the crown of a volcano in the sea) we found only two very old plants. On islands with feral goats or donkeys, such as Santiago and Baltra, we could only find plants on cliffs and other places inaccessible to these animals. In these cases, populations are formed by a few individuals, in many cases by a single plant. Most of the plants are very old, and new shoots sprout from the enlarged base of the stem.

Regarding coastal populations that were found on collecting expeditions from 1950 to 1974 and that are maintained in the TGRC, we found again populations in the north of Baltra, Bartolomé, north of Bahía James (in Santiago), and Corona del Diablo. However, despite exhaustive searches we found neither living plants nor dead remains of the previously reported *L. cheesmanii* populations on Santa Cruz (in the vicinity of the village of Puerto Ayora), Gardner, Pinzón, or Rábida. On the other hand, we discovered an important population not reported previously on Sombrero Chino Island.

Inland Santa Cruz yielded many *Eulycopersicon* populations (21), in many cases formed by hundreds of individuals spreading over tens of meters. In general, in contrast to the coastal populations, these populations are found in environments highly disturbed by humans. Most of the populations (18) corresponded to *L. esculentum* 'Gal cer,' while only three corresponded to *L. cheesmanii* 'long.' The few *L. cheesmanii* 'long' populations that we found occurred in the vicinity of or were intermingled with *L. esculentum* 'Gal cer.' On Santa Cruz Island, we also collected two samples of *L. esculentum* var. *cerasiforme*, present as a weed in vegetable gardens, and two accessions of cultivated *L. esculentum*.

AFLP analysis—A total of 593 fragments were scored, of which 534 (90.1%) were polymorphic. Of these, 222 were specific to subgenus *Eriopersicon*. Specific to subgenus *Eulycopersicon*.

lycopersicon were 112 fragments: of these 31 were only found in one or more accessions of *L. cheesmanii*, 21 were present only in *L. esculentum*, and 54 in *L. pimpinellifolium*, but none were limited to *L. esculentum* 'Gal cer.' When considering *L. cheesmanii* alone, 28 fragments were unique to *L. cheesmanii* 'short,' six to *L. cheesmanii* 'long,' but none were specific to *L. cheesmanii* f. *minor*. Within *L. esculentum*, we found 16 fragments in domesticated *L. esculentum* but not in *L. esculentum* var. *cerasiforme* and 45 fragments in *L. esculentum* var. *cerasiforme* but not in cultivated *L. esculentum*. Regarding fragments consistently present in all the accessions of one taxon of subgenus *Eulycopersicon* but not in any accession of the other taxa, we found one in *L. cheesmanii*, three in *L. pimpinellifolium*, but none in *L. esculentum* or any of its varieties.

Two fragments were shared by some *L. esculentum* 'Gal cer' and *L. esculentum* var. *cerasiforme* but not by the rest of members of the genus. One of these fragments was present in *L. esculentum* var. *cerasiforme* LA3123 coming from the Santa Cruz Island and also in all of the accessions of *L. esculentum* 'Gal cer.' Another fragment was present in the two *L. esculentum* var. *cerasiforme* from Santa Cruz (GLP-26 and LA3123) and in six of seven *L. esculentum* 'Gal cer' accessions. This shows that *L. esculentum* var. *cerasiforme* is genetically very similar to *L. esculentum* 'Gal cer.'

The total diversity (H_T) value for subgenus *Eriopersicon* ($H_T = 0.248$) was more than three times higher than that for *Eulycopersicon* ($H_T = 0.072$), indicating that the former is much more diverse than the latter (Table 4). Within subgenus *Eulycopersicon*, the highest gene diversity is found in *L. pimpinellifolium* ($H_T = 0.071$). *Lycopersicon esculentum* (domesticated plus *L. esculentum* var. *cerasiforme*) and *L. cheesmanii* have similar values (Table 4). In contrast, *L. esculentum* 'Gal cer' has a very low value for gene diversity ($H_T = 0.014$). Gene differentiation among *L. esculentum* var. *cerasiforme* (plus cultivated *L. esculentum*), *L. esculentum* 'Gal cer,' *L. pimpinellifolium*, and *L. cheesmanii* is the highest found in this work ($G_{ST} = 0.462$). When considering the three *L. cheesmanii* forms, the highest diversity is found within *L. cheesmanii* 'short' ($H_T = 0.060$) with a value almost twice that of *L. cheesmanii* 'long' and *L. cheesmanii* f. *minor*. These three forms have relatively low gene differentiation ($G_{ST} = 0.235$), although higher than the differentiation between subgenera *Eriopersicon* and *Eulycopersicon* ($G_{ST} = 0.190$). This low value is due to the huge variation within subgenus *Eriopersicon* more than to similarity between subgenera *Eriopersicon* and *Eulycopersicon*.

Genetic distances among the four groups in subgenus *Eulycopersicon* (Table 5) show that *L. esculentum* 'Gal cer' is very similar to the other *L. esculentum*. The highest genetic distance within this subgenus is between *L. cheesmanii* and *L. esculentum* 'Gal cer.' *Lycopersicon esculentum* 'Gal cer' is found at a greater genetic distance from *L. pimpinellifolium* than that separating this latter species from *L. cheesmanii* or even from *L. esculentum*.

Cluster analysis—Phenograms obtained from both Dice and Jaccard coefficients were highly congruent, as would be expected. The normalized Mantel statistic showed that both similarity matrices were highly correlated ($r = 0.986$; $t = 7.45$; $P > 0.999$).

The cluster analysis shows a clear separation between the much more divergent subgenus *Eriopersicon* and subgenus *Eulycopersicon* (Fig. 3). Within *Eulycopersicon*, four main

←

Fig. 2. Leaves and fruits of *Lycopersicon cheesmanii* 'short' (a), *L. cheesmanii* 'long' (b), *L. cheesmanii* f. *minor* (c), *L. esculentum* 'Gal cer' (d), and *L. esculentum* var. *cerasiforme* (e). Scales in centimeters. Note that scales are not represented at the same magnification factor.

TABLE 3. Main phenotypic traits (means \pm SE of the accessions evaluated) of wild Galápagos tomatoes (*Lycopersicon cheesmanii* f. *cheesmanii* 'short,' *L. cheesmanii* f. *cheesmanii* 'long,' *L. cheesmanii* f. *minor*, and *L. esculentum* 'Gal cer'), *L. pimpinellifolium*, and *L. esculentum* var. *cerasiforme* in a trial performed in a greenhouse in Valencia, Spain. Mean values for each accession were obtained as the mean of six plants, for which a variable number of flowers/fruits per plant was evaluated.

Traits	<i>L. cheesmanii</i> 'short'	<i>L. cheesmanii</i> 'long'	<i>L. cheesmanii</i> f. <i>minor</i>	<i>L. esculentum</i> 'Gal cer'	<i>L. esculentum</i> var. <i>cerasiforme</i>	<i>L.</i> <i>pimpinellifolium</i>
Number of accessions	4	3	9	7	3	11
Internode length (cm)	3.34 \pm 0.48	5.18 \pm 0.58	2.96 \pm 0.38	6.21 \pm 0.43	6.08 \pm 0.83	6.38 \pm 0.49
Petal length (cm)	7.98 \pm 0.74	8.68 \pm 0.56	9.43 \pm 0.57	9.34 \pm 0.27	9.52 \pm 0.64	10.82 \pm 0.56
Sepal length (cm)	3.43 \pm 0.64	4.40 \pm 0.63	5.27 \pm 0.40	3.56 \pm 0.25	4.34 \pm 0.82	4.01 \pm 0.16
Petal length/sepals length	2.44 \pm 0.30	2.11 \pm 0.48	1.99 \pm 0.24	2.65 \pm 0.16	2.33 \pm 0.35	2.72 \pm 0.14
Stamen length (mm)	5.88 \pm 0.44	6.99 \pm 0.30	6.90 \pm 0.13	7.14 \pm 0.16	7.60 \pm 0.40	7.65 \pm 0.26
Stigma exsertion (mm)	0.18 \pm 0.09	0.23 \pm 0.13	0.33 \pm 0.15	1.39 \pm 0.30	1.12 \pm 0.57	1.13 \pm 0.21
Fruit length (cm)	9.18 \pm 0.79	11.09 \pm 1.02	8.99 \pm 0.28	11.18 \pm 0.71	19.56 \pm 1.51	11.92 \pm 0.30
Fruit breadth (cm)	9.19 \pm 1.07	11.24 \pm 1.00	8.48 \pm 0.27	12.84 \pm 0.79	22.36 \pm 2.51	13.02 \pm 0.31
Soluble solids content ($^{\circ}$ Brix)	10.37 \pm 0.88	7.52 \pm 0.95	14.29 \pm 0.52	6.93 \pm 0.75	6.30 \pm 1.16	8.56 \pm 0.48
Flowering earliness (d)	122.3 \pm 43.6	104.0 \pm 37.9	175.0 \pm 11.7	28.0 \pm 0.3	30.3 \pm 1.4	59.3 \pm 12.8

clusters, supported by a bootstrap value of 99%, were formed by *L. cheesmanii*, *L. pimpinellifolium*, cultivated *L. esculentum*, and *L. esculentum* var. *cerasiforme* plus *L. esculentum* 'Gal cer.' A phenogram constructed using only *Eulycopersicon* accessions was very similar in topology to that obtained when using accessions of *Eriopersicon* and *Eulycopersicon* together.

Within the *L. cheesmanii* cluster, *L. cheesmanii* 'short,' *L. cheesmanii* 'long,' and *L. cheesmanii* f. *minor* were not completely separated into different subclusters. However, the tree shows that *L. cheesmanii* 'short' and *L. cheesmanii* 'long' vary more than *L. cheesmanii* f. *minor*; all accessions of the latter are grouped into the same cluster, which also includes one accession each of *L. cheesmanii* 'short' and *L. cheesmanii* 'long.' Within *L. pimpinellifolium* there are two subclusters, one for the Ecuadorian accessions and the other for the Peruvian accessions, with a bootstrap support of 70%. Within the branch that contains *L. esculentum* var. *cerasiforme* and *L. esculentum* 'Gal cer,' all the accessions of *L. esculentum* 'Gal cer' are situated in the same subcluster, separated slightly from *L. esculentum* var. *cerasiforme*. The distances among the different *L. esculentum* var. *cerasiforme* accessions, however, are greater than those among the *L. esculentum* 'Gal cer' accessions.

Principal components analysis (PCA)—The first PCA gave results very similar to those obtained with the cluster analysis. However, because the first component (61.6% of total variation) basically accounted for the separation from subgenus *Eulycopersicon* of subgenus *Eriopersicon*, a new PCA was performed including only *Eulycopersicon* accessions (Fig. 4). In this analysis, the first, second, and third components explain 28.5, 25.0, and 16.7%, respectively, of the total variation.

The graphic PCA (Fig. 4) clearly separates *L. cheesmanii* from *L. pimpinellifolium* from Ecuador or from Peru. Two *L. esculentum* var. *cerasiforme* accessions from Santa Cruz fall very close to *L. esculentum* 'Gal cer' group. The other *L. esculentum* var. *cerasiforme* accession from Isabela and the domesticated *L. esculentum* are somewhat more distant, although much less distant than either *L. pimpinellifolium* or *L. cheesmanii*. Regarding *L. cheesmanii*, it is not possible to make a clear separation, either among the different forms or among different geographical origins.

Correlations between geographical and genetic distances—Because no clear pattern of differentiation by geographical origin was observed from the cluster or PCA for the wild Galápagos tomatoes, within forms with a greater number of representatives (*L. cheesmanii* f. *minor* and *L. esculentum* 'Gal

TABLE 4. Gene diversity statistics (Nei, 1973) estimated from amplified fragment length polymorphism (AFLP) data for the genus *Lycopersicon*, subgenus *Eulycopersicon*, and *L. cheesmanii*.

Groups	Number of accessions	Number of polymorphic loci (%)	H_T^a	D_{ST}^a	H_S^a	G_{ST}^a
Genus <i>Lycopersicon</i>	43	534 (90.1)	0.210	0.040	0.170	0.190
Subgenus <i>Eriopersicon</i>	5	408 (68.8)	0.248			
Subgenus <i>Eulycopersicon</i>	38	242 (40.8)	0.072			
Subgenus <i>Eulycopersicon</i>	38	242 (40.8)	0.091	0.042	0.049	0.462
<i>L. esculentum</i> (incl. var. <i>cerasiforme</i>)	4	92 (15.5)	0.063			
<i>L. esculentum</i> 'Gal cer'	7	24 (4.0)	0.014			
<i>L. pimpinellifolium</i>	11	141 (23.8)	0.071			
<i>L. cheesmanii</i>	16	111 (18.7)	0.051			
<i>L. cheesmanii</i>	16	111 (18.7)	0.051	0.012	0.040	0.235
<i>L. cheesmanii</i> 'short'	4	88 (14.8)	0.060			
<i>L. cheesmanii</i> 'long'	3	42 (7.0)	0.031			
<i>L. cheesmanii</i> f. <i>minor</i>	9	48 (8.1)	0.028			

^a H_T = total gene diversity; D_{ST} = among-groups gene diversity; H_S = within-groups gene diversity; G_{ST} = relative magnitude of gene differentiation among groups (D_{ST}/H_T).

TABLE 5. Nei (1974) estimates of standard genetic distances (above the diagonal) and genetic identity (below the diagonal) between subgenus *Eriopersicon*, *Lycopersicon esculentum* (excluding ‘Gal cer’), *L. esculentum* ‘Gal cer,’ *L. pimpinellifolium*, and *L. cheesmanii*.

Taxon	<i>Eriopersicon</i>	<i>L. esculentum</i>	<i>L. esculentum</i> ‘Gal cer’	<i>L. pimpinellifolium</i>	<i>L. cheesmanii</i>
<i>Eriopersicon</i>		0.167	0.202	0.162	0.162
<i>L. esculentum</i>	0.846		0.042	0.073	0.079
<i>L. esculentum</i> ‘Gal cer’	0.890	0.958		0.099	0.116
<i>L. pimpinellifolium</i>	0.850	0.930	0.905		0.095
<i>L. cheesmanii</i>	0.850	0.924	0.890	0.910	

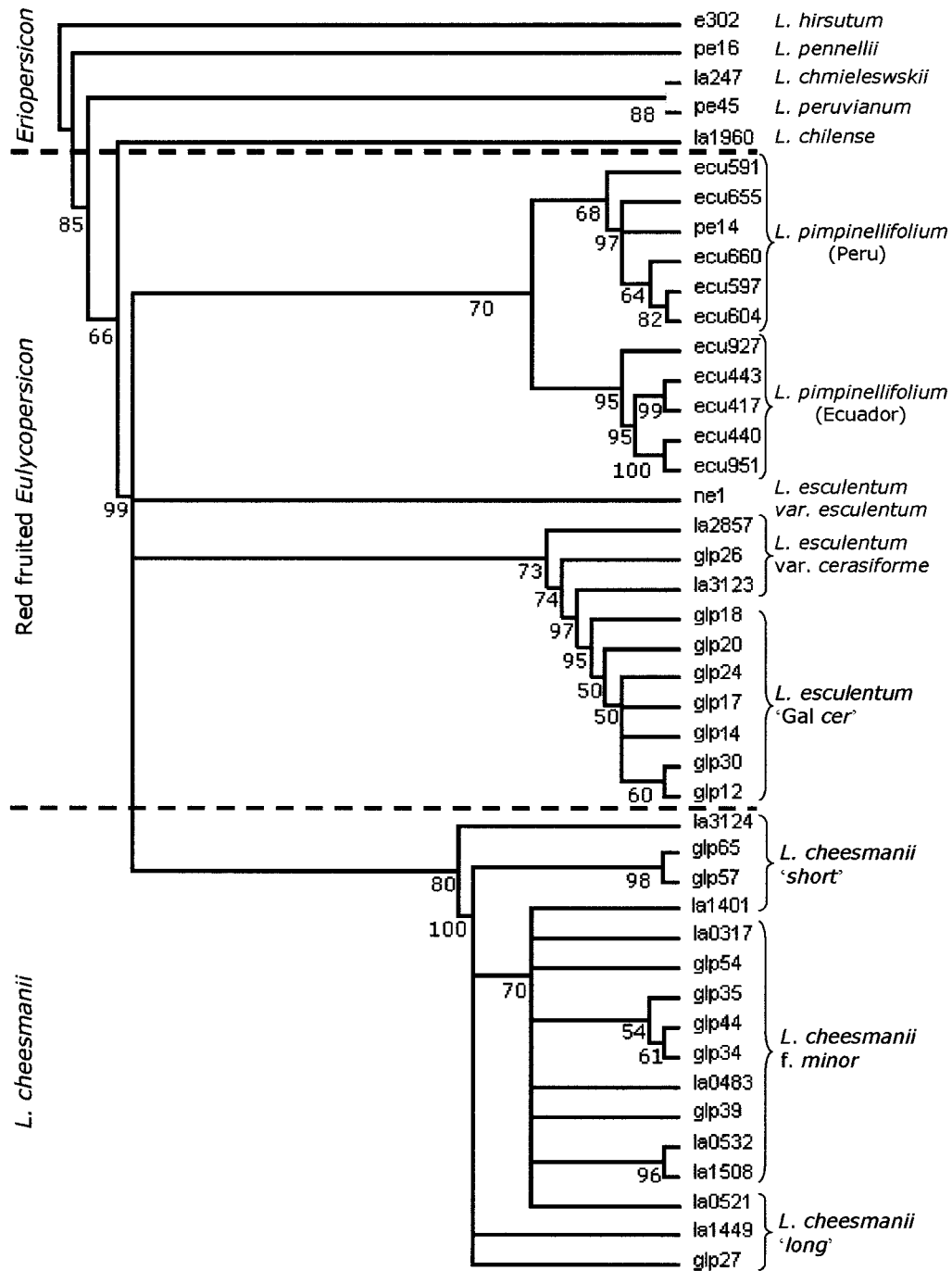


Fig. 3. UPGMA phenogram of 43 accessions of genus *Lycopersicon* based on AFLPs. Phenetic relationships were derived from Dice AFLP-based pairwise genetic distances. Bootstrap values (percentages; 1000 replications) are indicated at each node. Only nodes with a bootstrap value >50% have been represented. Dashed lines indicate the separation between subgenus *Eriopersicon*, red-fruited *Eulycopersicon*, and *L. cheesmanii*.

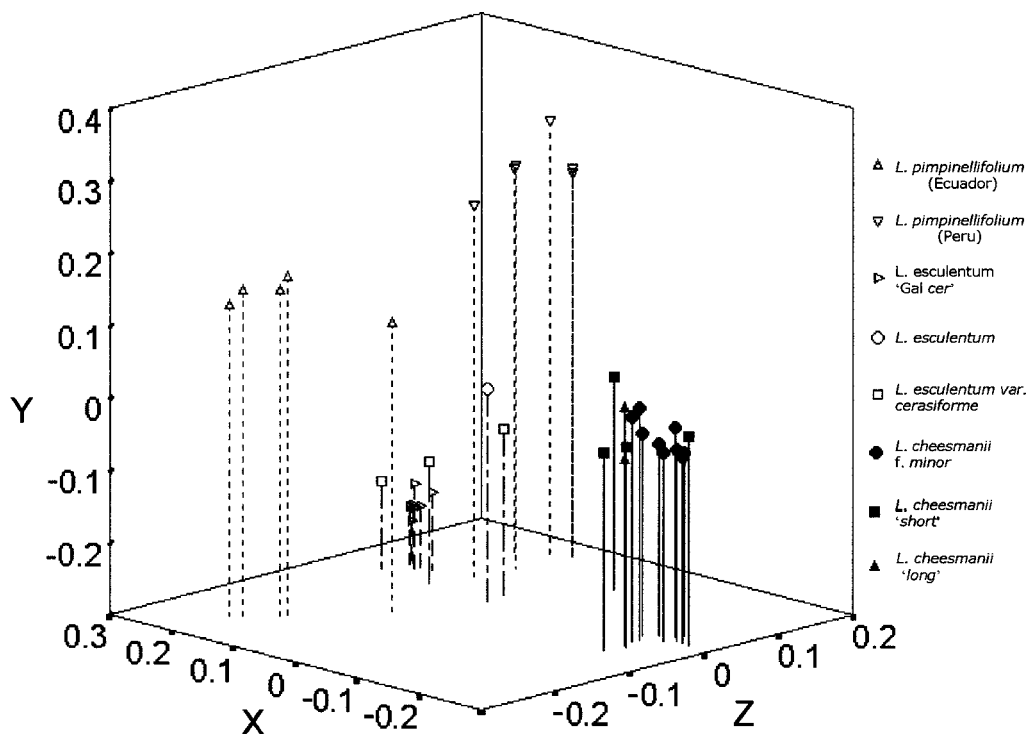


Fig. 4. Principal components analysis plot of 38 *Eulycopersicon* accessions for the three principal components (X, Y, Z) estimated from the AFLP similarity matrix.

cer') we studied the correlation between geographic and genetic distance between pairs of accessions. In neither case did we find a significant correlation between the two variates, indicating that there is no relationship between the geographic distance that separates the place of origin of each accession and the genetic distance between them (Fig. 5).

DISCUSSION

Relationships of Galápagos tomatoes—*Lycopersicon cheesmanii* differs from the rest of *Eulycopersicon* species by a set of traits and can be clearly distinguished from them by one or more of the following gross morphological traits: leaf subdivision (three- to four-pinnate leaves), internode length (short internodes; 2–4 cm), and fruit color (pale straw-yellow to orange-red). Furthermore, *L. cheesmanii* does not flower in winter under our conditions, while the other *Eulycopersicon* species do. In addition, coastal populations (*L. cheesmanii* 'short' and *L. cheesmanii* f. *minor*) have a slow and difficult germination if seeds are not treated.

At the molecular level, the Galápagos *L. cheesmanii* can be unambiguously separated from the other species of the genus. However, there is no clear molecular distinction among the three *L. cheesmanii* forms that we have considered here, indicating that all native Galápagos tomatoes (*L. cheesmanii*) share a common genetic background. This supports the idea of Rick (1983) and R. Chetelat (TGRC, personal communication) that the heterogeneity for many traits among the different accessions of *L. cheesmanii* does not permit a rational classification into genetically differentiated subgroups. Perhaps a few genes are responsible for the gross morphological differences (leaf subdivision and internode length) among the forms we have considered. For instance, the highly dissected

leaf trait characteristic of *L. cheesmanii* f. *minor* is controlled by a single gene (Rick, 1980). In addition, some point mutations result in short internode phenotypes (Zeewart, 1984; Nadzhimov et al., 1988), a trait that distinguishes *L. cheesmanii* 'short' and *L. cheesmanii* f. *minor* from *L. cheesmanii* 'long.' Nonetheless, these forms have important differences in some traits, such as soluble solids concentration, that have a complex inheritance (Saliba-Colombani et al., 2001) and that are very interesting for tomato breeding. Therefore, at least from a breeders' point of view, separating these three forms is of clear utility.

Lycopersicon esculentum 'Gal cer' is morphologically and genetically very similar to *L. esculentum* var. *cerasiforme*. However, the fruit size is much smaller than that of typical *L. esculentum* var. *cerasiforme* and similar to *L. pimpinellifolium*. In this respect, we agree with the observation of R. Chetelat (TGRC, personal communication) that fruit size alone should not be used to distinguish between red-fruited *Eulycopersicon* taxa. Because the diameter of the fruit of *L. esculentum* var. *cerasiforme* and *L. pimpinellifolium* may overlap and is subject to environmental influence, a combination of traits should be used instead.

Origin of Galápagos tomatoes—The molecular and morphological analyses show that, as Rick (1956) proposed and subsequent studies confirmed, *L. cheesmanii* is indeed a member of the subgenus *Eulycopersicon* (Palmer and Zamir, 1982; Miller and Tanksley, 1990; Bretó et al., 1993; Williams and StClair, 1993; Alvarez et al., 2001; Marshall et al., 2001; Peralta and Spooner, 2001; Nesbitt and Tanksley, 2002). *Lycopersicon cheesmanii* shares most molecular markers with *L. pimpinellifolium* and *L. esculentum*, i.e., they are obviously

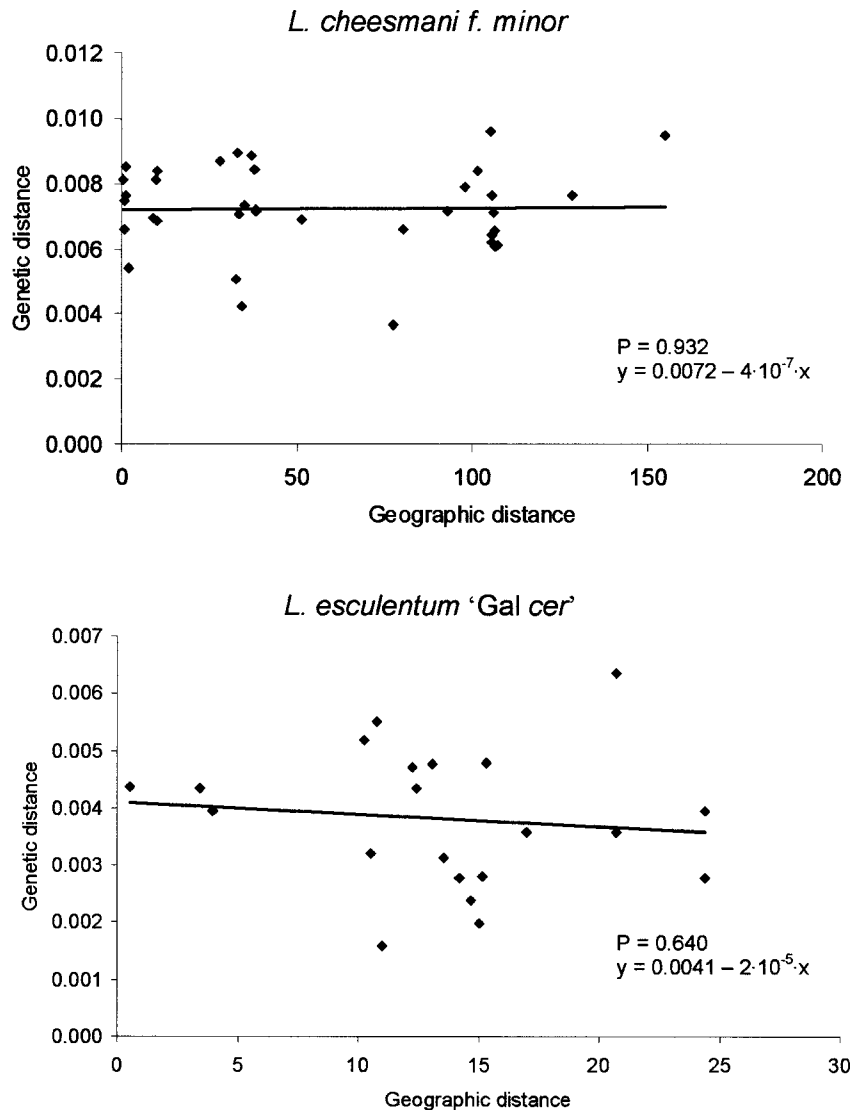


Fig. 5. Relationships between geographic distance (in kilometers) and Dice pairwise genetic distance for *Lycopersicon cheesmanii* f. *minor* and *L. esculentum* 'Gal cer.'

sister species and therefore appropriately placed in the same subgenus. The fact that genetic diversity within *L. cheesmanii* is almost as great as that found in *L. pimpinellifolium* suggests that *Lycopersicon* migrated to the Galápagos some time ago. It is unknown when the successful establishment that resulted in the present day *L. cheesmanii* occurred. However, Nesbitt and Tanksley (2002) suggest, on the basis of DNA sequences, that the three *Eulycopersicon* species (*L. cheesmanii*, *L. esculentum*, and *L. pimpinellifolium*) diverged from a common ancestor approximately one million years ago. Therefore, this is the minimum limit for the establishment of the *Eulycopersicon* that resulted in the present day Galápagos *L. cheesmanii*. Because *L. cheesmanii* f. *minor* is characterized by a derived trait (conferred by gene *Pts*), it is probably more recent in origin. *Lycopersicon cheesmanii* f. *minor* is adapted to a very specific environment, which would account for its lower diversity despite its relatively greater abundance. Whether the *Pts* gene is directly involved in the greater adaptation to coastal conditions or whether it has simply become fixed as a result of inbreeding remains to be determined.

Regarding *L. esculentum* 'Gal cer,' the lack of references to this type of tomato in previous studies (Rick, 1956, 1963), and its low genetic diversity suggest an extremely recent origin. Although their fruit size is similar to *L. pimpinellifolium*, the molecular markers for *L. esculentum* 'Gal cer' are very different from the accessions of *L. pimpinellifolium* we used. In fact, the genetic distance of *L. pimpinellifolium* from *L. esculentum* 'Gal cer' is greater than that separating *L. pimpinellifolium* from *L. cheesmanii*, two very clearly different species. Our work suggests that *L. esculentum* 'Gal cer' appeared after introduction and naturalization of *L. esculentum*. The presence of the weedy *L. esculentum* var. *cerasiforme* has been documented in the inhabited island of Santa Cruz since 1952 (Rick, 1956). R. N. Lester (University of Birmingham, personal communication) suggested that natural selection by frugivorous animals might have exerted a strong selection pressure favoring a reduction in the fruit size of *L. esculentum* var. *cerasiforme*, which would have resulted in the *L. esculentum* 'Gal cer' form.

Present distribution and genetic erosion of Galápagos tomatoes—Isolation from the continent and the singular ecological and climatological conditions under which the Galápagos tomatoes have evolved give them a high value both as genetic resources for the improvement of cultivated tomato and for the study of the evolution of an island endemic from continental ancestors. Unfortunately, the recent introductions of animals and plants, as well as human pressures, have put the endemic Galápagos *L. cheesmanii* at risk of extinction.

We located populations of Galápagos tomatoes conforming to *L. cheesmanii* and *L. cheesmanii* f. *minor* (Rick, 1963). Because intrapopulation variation in these forms is negligible (Rick and Fobes, 1975) but interpopulation variation is relatively high, extinction of local populations results in the irreversible loss of genetic material. Several populations reported from the 1950s to the 1970s have not been found since then despite exhaustive searches. Other populations have been rediscovered, but in some cases, they are endangered; e.g., in Santiago, *L. cheesmanii* plants are found only in areas out of reach of feral goats and donkeys, and the populations usually consist of a few old plants. In other cases, such as in the vicinity of the village of Puerto Ayora in Santa Cruz, where several populations were previously reported (Mackinney et al., 1954; Rick, 1956), the increase in human activity has probably been responsible for their complete disappearance. Our observations point to feral goats and donkeys and expanded human activity (e.g., habitat loss) as the main factors responsible for elimination of individual populations. We have found only one population (on Sombrero Chino) not reported previously, which is further evidence of elimination of populations, particularly coastal forms.

Some inland populations on Santa Cruz, which we have called *L. esculentum* 'Gal cer,' are made up of many individuals, even hundreds in some cases. These *L. esculentum* 'Gal cer' populations, not previously described, are found in highly disturbed areas, on occasions intermingled with *L. cheesmanii* 'long.' Our observations suggest that native *L. cheesmanii* 'long' could be displaced by this recently introduced *L. esculentum* 'Gal cer,' because *L. esculentum* 'Gal cer' is a highly efficient invader of disturbed open spaces that produces large quantities of fruits and seeds dispersed by different animals, including the Galápagos mockingbird.

In addition, all the members of *Eulycopersicon* can intercross and the offspring is fertile, suggesting that they have not completed the speciation process that results in crossing barriers. Although we did not find direct evidence in our data, it is still possible that *L. cheesmanii* 'long' populations have already suffered a loss of genetic integrity through hybridization with *L. esculentum* 'Gal cer,' because both taxa can be found intermingled or physically very close. Although *L. cheesmanii* has inserted or weakly exerted stigmas, which leads to autogamy (Rick, 1963), styles of *L. esculentum* 'Gal cer' are longer and the stigmas are exerted, thus some cross-pollination is likely (Rick et al., 1978). In addition, the native Galápagos bee, *Xylocopa darwinii*, has been observed by Rick (1963) to visit *L. cheesmanii* flowers and by us to visit the *L. esculentum* 'Gal cer' flowers. This suggests that occasional hybridization between adjacent *L. cheesmanii* 'long' and *L. esculentum* 'Gal cer' is possible, although it would probably favor pollen transfer from *L. cheesmanii* to *L. esculentum*.

Conservation considerations—The lack of correlation between genetic and geographic distances in *L. cheesmanii* prob-

ably is a consequence of the strict autogamy throughout the species, leading to rapid, and often fortuitous fixation of alleles (genetic drift) and differentiation between populations, even if they are physically very close (Rick, 1963, 1983). In fact, the genetic distances among the four *L. cheesmanii* f. *minor* populations from the small island of Bartolomé are similar to the genetic distances among other *L. cheesmanii* f. *minor* populations from other islands. This means that conservation of substantial diversity might be achieved if a few protected areas were established where several populations are found. It also has important implications for conservation ex situ, because high diversity could be collected by visiting just a few places where many scattered populations exist. Furthermore, within *L. cheesmanii*, the greatest DNA diversity was found in *L. cheesmanii* 'short' and the lowest in *L. cheesmanii* f. *minor*. Rick and Fobes (1975) also found that *L. cheesmanii* f. *minor* was the least variable form. This indicates that most genetic variation could be conserved by preserving the more variable forms of *L. cheesmanii*.

Because an important part of the great DNA diversity can be found in a single area, establishment of a few protected areas could enable the conservation of most of the natural diversity of native Galápagos tomatoes. These areas should include protection of the native populations from introduced herbivorous animals and removal of all *L. esculentum* 'Gal cer' plants from the surroundings. Collecting Galápagos tomatoes in order to conserve them ex situ can also provide a means to prevent the irreversible loss of germplasm. In this way, accessions that were collected from the 1950s to the 1970s by C. M. Rick and others could be useful to reestablish some extinct natural populations.

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