Pl. Syst. Evol. 132, 279—298 (1979)

Plant Systematics and Evolution

From the Department of Vegetable Crops,
University of California, Davis

Evolution of Mating Systems in *Lycopersicon hirsutum* as Deduced from Genetic Variation in Electrophoretic and Morphological Characters

By

Charles M. Rick, Davis, **Jon F. Fobes,** East Lansing,
and **Steven D. Tanksley,** Davis

(Received August 8, 1978)

Key Words: *Lycopersicon hirsutum, Solanaceae.*—Allozymes, clinal variation, electrophoresis, evolution of mating systems, isozymes, self-incompatibility.

Abstract: Populations in the central part of the distribution are mostly self-incompatible and tend to be highly variable for allozymic and morphological characters; those in the north and south limits are entirely self-compatible and tend to be genetically highly uniform. Gradations in variability are observed in the intermediate regions. Flower size tends to diminish in the peripheral areas. The extensive differences in genotype observed between the north and south marginal populations are not compatible with the concept of a single origin of self-compatibility, but suggest, along with other evidence, that the substitution of different alleles resulted from differentiation in the marginal areas from older, self-incompatible stocks of the central region. The conclusions regarding patterns of genetic variation and nature of evolution of mating systems in *L. hirsutum* conform to a remarkable extent with those reached previously for *L. pimpinellifolium*, a species that is distinct in morphology and ecological preferences yet has a similar latitudinal distribution.

Amongst the tomato species, *Lycopersicon hirsutum* HUMB. & BONPL. is the most robust in growth and develops the showiest floral displays; high rates of insect cross-pollination might therefore be expected. In this respect it is the antithesis of the diminutive and smaller flowered *L. pimpinellifolium* (JUSL.) MILL., a species with similar geographic distribution. They are almost identical in latitude range but differ in their elevation preferences in western Ecuador and Perú. The latter grows in coastal areas, the former prefers higher elevations (1,500-3,000 m) in the

0378-2697/79/0132/0279/$ 04.00

Andean valleys, except in Ecuador where it also spreads into the coastal lowlands and is sympatric with the former.

A study of genetic variation in *L. hirsutum* should take note of the subspecific taxon f. *glabratum* (C. H. Mull.)[1]. Muller (1940) delinates this variant in terms of a general reduction in hairiness of stems and leaves, very much smaller (2 cm) corolla, and more slender calyx, but, observing intergrades with the typical form, relegates it to the category of a form[1]. In our study of the species, we could not fail to be impressed with the distinctness of the Ecuadorean accessions in respect to their smaller flower size, more diminutive plant parts, and general reduction in hairiness. In the available accessions, intergradation with the typical form appears only in the Huancabama region of Perú, where the unique, monogenically determined, qualitatively distinct *h* variant exists. Although large epidermal hairs are reduced to a greater extent in the latter biotypes than in Ecuadorean *glabratum*, they do not fit Muller's concept of *glabratum* in respect to flower size, calyx, or other characteristics. In view of this confusion in concepts and misidentification of many herbarium specimens, we shall not attempt to distinguish any subspecific taxa, but refer only to Ecuadorean vs. Peruvian *hirsutum*.

Self-incompatibility of the Peruvian *hirsutum* was discovered by McGuire and Rick (1954) and its distribution and inheritance were investigated by Martin (1963). The species has been receiving considerable attention for its potential value as a source of genes for disease resistance and particularly for insect resistance, possibly conditioned by the strong, unpleasant (to most human subjects) pungency of its herbage (Stoner 1970 and unpublished).

Our study of allozyme variability in *L. pimpinellifolium* (Rick & al. 1977) revealed immense differences in genetic variability as measured by several criteria between the observed maxima in NW. Perú and the minima in the northern and southern margins of its range. High positive correlations were determined between the extent of genetic variability, rates of cross-pollination (CP), flower size, and degree of stigma exsertion. Our subsequent study of cross-pollination in a test plot in S. Perú (Rick & al. 1978) ascertained that the relationships were stronger with flower size than with stigma exsertion, and demonstrated that interregional differences in CP were influenced less by population dynamics of the pollinating bees and other environmental factors than by hereditary variability in floral traits of the host plant. On the basis

---

[1] *Lycopersicon hirsutum* Humb. & Bonpl. f. *glabratum* (C. H. Mull.) Rick, Fobes & Tanksley.—Basionym: *L. hirsutum* var. *glabratum* Muller, U.S. Dept. Agric. Misc. Publ. 382 (1940).

of these facts, the observed patterns of variability in *L. pimpinellifolium* could be interpreted according to the following rationale. The region of highest variability roughly approximates the hypothetical region of origin, high variability and outcrossing being characteristic of the ancestral species and being selectively advantageous in that region of great ecological diversity. As the species spread north and south, variability diminished as a result of genetic drift and selection for a limited number of highly successful, prolific genotypes. Fixation of the latter genotypes was expedited by selection for floral structure modifications that promote inbreeding.

The aforementioned observations and deductions stimulated a similar study of *L. hirsutum* to ascertain to what extent if any similar relationships might hold in a species of nearly identical geographic distribution, yet radically different morphology and natural breeding system. As mentioned above, the flowering of *L. hirsutum* is vastly more showy than that of *L. pimpinellifolium*. The two species also differ markedly in respect to the heavy, robust character of the former in contrast to the dainty, slender parts of the latter, radical differences in foliage volatiles, and other physiological characteristics. Possibly the most profound difference affecting population structure is the prevailing self-compatibility of *L. pimpinellifolium* and the aforementioned self-incompatibility of the majority of the *hirsutum* accessions.

Materials and Methods

Our study was made on 52 living accessions of *L. hirsutum* scattered over the entire known range of the species (Table 1, Fig. 2). Voucher specimens are kept in herbarium of C. M. Rick.—This material suffers the following shortcomings for a study of genetic variability: 1) a few collections were made without adequate records; 2) seeds of certain accessions have been regenerated in various, often unspecified, procedures since the original collections were made; and 3) natural populations tend to be small (each often limited to five plants or less). Sampling was further limited by the lack of ripe fruit on certain plants. Acknowledging such shortcomings, we nevertheless deemed it sufficiently important to conduct the investigations, since future opportunities for acquiring superior material are highly uncertain. The available information concerning the number of wild plants sampled, the generation of the seeds used in the survey (when known), and other pertinent population parameters is presented in Table 1.

General observations were made on gross morphology of at least one culture of each accession. Except for the monogenic hair character *h*, these data are not included in Table 1.

Allozyme variation for acid phosphatase (*Aps*), esterase (*Est*), glutamate oxaloacetate transaminase (*Got*) and peroxidase (*Prx*) systems was analyzed by standard methods for horizontal slab starch-gel electrophoresis. Details concerning the methodology are given by Rick & Fobes (1975a), Rick & al. (1976), and Rick & al. (1977). A standard number of sixteen individuals was adopted for sampling the progeny of each wild plant.

Empirical improvements in the esterase activity stain revealed a number of new banding phenotypes controlled by several loci in addition to *Est-1*. These improvements included: 1) using α-naphthyl butyrate as the enzyme substrate replacing previously used α-naphthyl valerate and 2) modifying the pH of the staining solution to pH 6.0 using 0.1 M sodium phosphate buffer. α-naphthyl butyrate was prepared as a 1% solution in 100% acetone and used at a rate of 5 ml per 100 ml staining solution. The staining salt was Fast Blue RR Salt (Sigma) at 0.1 g per 100 ml solution. It was also possible to analyze variation at two phosphoglucomutase (*Pgm*) loci utilizing the following staining recipe: 350 mg glucose-1-phosphate (disodium salt); 8 mg NADP; 20 mg MTT; 1.5 ml $MgCl_2$ (1% aqueous sol.); 5 mg PMS; 35 units glucose-6-phosphate dehydrogenase; 75 ml 0.1 M Tris, pH 8.0. The results of these additional tests are not presented in Table 2 but are mentioned in appropriate places in the *Results* section because it was not possible to apply them to all accessions.

Reaction to self-pollination was determined for all accessions. Six or more flowers on each of six or more plants per accession were artificially self-pollinated in an insect-free greenhouse. Sib pollinations were made as controls. The proportion of flowers setting fruit could be read accurately two weeks after pollination, and relative seed yields were apparent two months later.

In the course of our collecting *L. hirsutum*, we made observations of the insect pollinators of this species. Unfortunately, travel schedules seldom allowed opportunity to visit each wild population at the optimum time for such purposes—in fact, in the majority of sites no such activity was observed.

Self-Incompatibility

Reactions to the pollinations to test self-compatibility reactions were generally decisive and fell into one of two categories—either the selfs failed to set any fruit or they resulted in fruit setting and seed production equivalent to those of sib pollinations. This clear-cut difference permitted simple classification into self-compatible (SC) or self-incompatible (SI) categories, as indicated in Table 1. Whereas it was known previously that the Ecuadorean accessions are self-compatible, our survey also revealed that the southernmost populations can also reproduce by self-pollination. Further aspects will be discussed later in relation to population variability.

Natural Pollination

Our collective observations on natural pollinators of *L. hirsutum* suggest that the time of greatest activity is in the mid- to late morning, corresponding to that for *L. pimpinellifolium* (Rick & al. 1978). When it was possible to visit populations at that time (LA 1264, 1295, 1347, and other sites from which viable seeds were not available), the intensity of bee visitations was impressive. Flowers of each plant in the population were visited by a wide assortment of species of Bombinid, Andrenid, Colletid, Meleponid, and Xylocopid bees. From the array of pollen types seen on the collected specimens it appeared that the majority of

bees were foraging different plant species in polylectic fashion. Since the data thus collected were meagre and could be taken from only a small fraction of the total populations collected, the details are not presented here.

Electrophoretic Characters

Rationale. Electrophoretic variation in this species will be characterized as explained below. In contrast to our previous studies on *L. cheesmanii* (RICK & FOBES 1975a), *L. esculentum* (RICK & FOBES 1975b), the "*minutum* complex" (RICK & al. 1976), and *L. pimpinellifolium* (RICK & al. 1977), the inheritance of zymotypic variation was not studied in progenies from controlled crosses within *L. hirsutum* or in crosses with standard *esculentum* lines in order to clarify inheritance of variations. It was nevertheless clear from certain progenies of wild plants that the variant bands seen represented alternate alleles at a given locus. The results clearly demonstrated that, with possible exceptions in peroxidases, the loci bands appear in the same positions in *L. hirsutum* as they do in the aforementioned species. The exceptions in peroxidases appear to be additional loci in *L. hirsutum*. Inasmuch as alleles could not be matched confidently with those of the other species (except for the normal *esculentum* + alleles represented as controls on every gel), the variants are arbitrarily designated by positions with reference to the + bands. Thus, an allozymic band that is advanced 5 mm beyond the position of the + band is labelled "a5"; one that is retarded 2 mm, "r2", etc. In this fashion we are not committing ourselves to the exact identification of alleles. This conservative attitude is also maintained in regard to allozymes of very similar phenotype: we do not distinguish between alleles unless positions differed by at least 2 mm or other strong evidence of phenotypic difference obtained.

This system is unambiguous for all loci except *Prx-4*. According to our previous experience (RICK & FOBES 1976), this locus codes for a complex of bands in both anodal and cathodal arrays. Accordingly we have applied arbitrary capital letter symbols to most of the variant alleles of this locus, the key to which appears in Fig. 1. Here again, we do not recognize new alleles unless their gel phenotypes are unequivocally different. This conservative attitude undoubtedly results in the neglect to a certain extent of the naturally occurring variability. A more extensive analysis of segregating generations within *L. hirsutum* and between it and standard *esculentum* lines would no doubt aid in delineating additional alleles, and application of more refined electrophoretic techniques would probably uncover more variation, as it

Table 1. Summary of collection data, compatibility reaction,

Access. No.	Site of collection	Dept. or province
Ecuador		
LA 1624	Jipijapa	Manabí
1625	Jipijapa	Manabí
407	Guayaquil: El Mirador	Guayas
1264	Bucay	Chimborazo
1266	Pallatanga	Chimborazo
1223	Alausí	Chimborazo
1255	Loja: Pedestal	Loja
1252	Loja: Jardín Botanico	Loja
1253	Pueblo Nuevo	Loja
Peru		
1738	R. Piura: Desfiladero	Piura
1739	W. of Canchaque	Piura
1737	Cashacoto	Piura
1736	Pucutay	Piura
1740	W. of Huancabamba	Piura
1717	R. Huancabamba: Sapalache	Piura
1718		Piura
1741	Sondorillo	Piura
1391	R. Utcubamba: Corral Quemado	Amazonas
386	Cajamarca: Inca Baths	Cajamarca
387	Santa Apolonia	Cajamarca
1352	R. Jequetepeque: Rupe	Cajamarca
1353	Contumazá	Cajamarca
1354	R. Chicama: N. of Cascas	Cajamarca
1347	R. Moche: Empalme Otusco	La Libertad
1775C	R. Casma: 61 km fr. Carr. Panam.	Ancash
1361	Pariacoto	Ancash
1775F	71 km fr. Carr. Panam.	Ancash
1362	Chacchan	Ancash
1780	76 km fr. Carr. Panam.	Ancash
1779	85 km fr. Carr. Panam.	Ancash
1778	92 km fr. Carr. Panam.	Ancash
1777	97 km fr. Carr. Panam.	Ancash
1393	97 km fr. Carr. Panam.	Ancash
1366	R. Fortaleza: Cajacay	Ancash
1298	R. Chillón: Yaso	Lima
1761	E. of Yaso	Lima
1559	Empalme Huamatanga	Lima
94	92 km from Lima	Lima
1764	W. of Canta	Lima
1772	W. of Canta	Lima
1558	Canta	Lima
1295	R. Rímac: Surco	Lima

and hair type for accessions of *L. hirsutum*

Elevation (m)	Collectors	Source	No. wild plants sampled	Compat. reaction	Hair type
100	RC, HW	Orig.	?	SC	+
100	RC, HW	Orig.	?	SC	+
50	CR, MR	Incr.	1	SC	+
400	CR, MR	Orig.	5	SC	+
800	CR, MR	Orig.	1	SC	+
1,800	TG, CR, MR	Orig.	3	SC	+
2,000	CR, MR	Orig.	3	SC	+
2,200	CR, MR	Orig.	2	SC	+
1,800	CR, MR	Orig.	2	SC	+
900	CO	Orig.	?	SI	+/*h*
1,100	RC, HW	Orig.	?	SI	*h*
1,900	CO	Orig.	?	SI	+/*h*
2,000	CO	Orig.	?	SI	+/*h*
2,200	RC, HW	Orig.	?	SI	+/*h*
2,500	RC, HW	Orig.	1	SI	+
2,500	RC, HW	Orig.	?	SI	+
1,600	HW, RC	Orig.	?	SI	+
1,000	MH	Incr.	?	SI	+
	CR, MR	Incr.	2	SI	+
	CR, MR	Incr.	1	SI	+
2,100	CR, MR, EV	Orig.	1	SI	+
2,650	MR, CR, EV	Orig.	3	SI	+
2,300	CR, MR	Orig.	1	SI	+
2,100	MR, CR, EV	Orig.	3	SI	+
1,400	JF, EV	Orig.	1	SI	+
1,500	CR, MR	Orig.	3	SI	+
2,100	JF, EV	Orig.	1		+
2,200	CR, MR	Orig.	3	SI	+
2,300	JF, EV	Orig.	1		+
2,700	JF, EV	Orig.	1		+
3,000	JF, EV	Orig.	3		+
3,200	JF, EV	Orig.	2		+
3,200	MH	Incr.	?	SI	+
2,300	CR, MR	Orig.	3	SI	+
1,600	CR, MR	Orig.	5	SI	+
1,700	JF, EV	Orig.	1	SI	+
2,200	DB	Orig.	2	SI	+
2,500	CR, MR	Incr.	1	SC	+
2,400	JF, EV	Orig.	4	SI	+
2,600	JF, EV	Orig.	3	SI	+
2,700	DB	Orig.	1	SI	+
2,000	CR, MR, EV	Orig.	1	SI	+

Table 1 (continued)

Access. No.	Site of collection	Dept. or province
1753	70 km fr. Lima	Lima
1560	Matucana	Lima
1745	R. Cañete: Putinza	Lima
1696	Huanchuy-Cacra	Lima
1695	Cacachuhuasin	Lima
1681	Mushka	Lima
1691	Yauyos	Lima
1730	R. San Juan: 78 km fr. C. Panam.	Huancavelica
1731	86 km fr. C. Panam.	Huancavelica
1721	R. Pisco: Ticrapo Viejo	Huancavelica

DB = David Baumann, RC = Raymond Clark, JF = Jon Fobes, TG = Tomas Guerrero, MH = Miguel Holle, DN = Daniel Nakama, CO = Carlos Ochoa, CR = Charles Rick, MR = Martha Rick, EV = Eduardo Vallejos, HW = Harold Winters.

did in the studies of Singh (1976) and others on genetic variation in *Drosophila* species.

The only new locus recognized in this study, designated *Prx-3b*, is expressed phenotypically by bands retarded at least 5 mm from the position of the normal allele of *Prx-3*. We feel reasonably confident about this decision because variation at the two positions seems to be independent. Other bands that might be coded by additional loci appear in positions more accelerated than *Prx-3* and between *Prx-3* and *Prx-4*. Until sufficient evidence for separate loci has been obtained, we prefer to treat these groups conservatively as alleles of *Prx-3* and *Prx-4* respectively; at any rate these variations have virtually no impact on the results or conclusions.

Refinements in our procedures for esterases, outlined in the section on *Methods*, resulted in consistent staining of additional bands, representing at least five new loci not previously detected in other species. Since these improvements were effected during the course of the *hirsutum* investigations, they could not be applied to all accessions. Further, our experience with these variations has been too limited to discriminate between alleles and loci. For these reasons, such data are not included in Table 2, but are presented briefly in appropriate places below.

Keeping in mind the aforementioned limitations, let us consider the nature of the results, which are summarized in Table 2. Regarding a genotypic characterization of the species as a whole, rather few

Table 1 (continued)

Elevation (m)	Collectors	Source	No. wild plants sampled	Compat. reaction	Hair type
2,100	JF, EV	Orig.	2	SI	+
2,400	DB	Orig.	1	SI	+
1,800	JF, EV	Orig.	2	SC	+
2,100	DB	Orig.	1	SC	+
1,900	DB	Orig.	1	SC	+
2,450	DB	Orig.	1	SC	+
2,900	DB	Orig.	1	SC	+
1,700	EV	Orig.	2	SC	+
2,100	EV	Orig.	4	SC	+
2,100	DB, MH, DN	Orig.	?	SC	+

Orig. = Progeny from seed collected in wild; Incr. = from seed increased in culture; SC = self-compatible; SI = self-incompatible; $+/h$ = variable hairiness; + = uniformly hairy.

generalizations can be drawn. Variability between and within populations and particularly the replacement of alleles from one end of the distribution to the other (considered below) are some of the problems of the dynamically evolving system that obstruct such a characterization. The following prevailing alleles appear to be distinctive in *L. hirsutum*: *Aps-1*^{r2}, *Est-1*^{a2}, and *Got-4*^{a8}, ^{a22}, and ^{a24}. Most the the *Prx-4* alleles are also distinctive, but too numerous for any single allele to typify the species (Fig. 1, Table 2). Insofar as our investigations of the tomato species have progressed, these alleles seem to distinguish *L. hirsutum* from the others. The electrophoretic data are therefore consistent with those of gross morphological characters in underscoring the distinctiveness of *L. hirsutum*.

Extent of Variability. Questions concerning the kinds of alleles in various parts of the distribution will be confronted below. The results of the survey are summarized in Table 2 in approximate order from north to south, and, within a given latitude (from west to east, in Perú mostly along river drainages since they provide the only habitats).

The degree of variability is expressed simply in terms of the proportion of loci that are polymorphic per accession. For the following reasons the data do not permit the derivation of such other, more precise measures as per cent heterozygosity or frequency of alleles per locus, etc. in each accession. 1) The small number or unknown number of plants per population is unsatisfactory for estimating such parameters. 2) Insufficient progeny plants were sampled to justify esti-

Table 2. Summary of kinds of allel

Access. No.	*Aps-1*	*Aps-2*	*Est-1*	*Got-1*	*Got-2*	*Got-3*	*Got-4*
Ecuador							
LA 1624	~	~	*a2*	+	*a20*	+	*a8*
1625	~	~	*a2*	+	*a20*	+	*a8*
407	*r4*	+	*a2*	+	*a20*	+	*a8*
1264	*a5*	+	~	+	*a20*	+	*a8*
1266	~	+	~	+	*a20*	+	*a8*
1223	*r2*	+	*a2*	+	*a20*	+	*a8*
1255	*r2*	+	*a2*	+	*a20*	+	*a8*
1252	~	+	~	*a2*	*a20*	+	*a8*
1253	~	+	~	*a2*	*a20*	+	*a8*
Peru							
1738	~	~	*a2*	+	*a15*	+	*a8*
1739	~	~	*a2*	+	*a10*	+	+
1737	~	~	+, *a2*	*a2*	*a10, a15*	+	+
1736	~	~	*a2*	+	*a15?*	+	*a8*
1740	~	~	*a2*	+, *a2*	*a10, n*	+	*a8, a22*
1717	+, *n*	+, *n, a4*	+, *r4*	+, *n*	*a20*	+	*a8, a22*
1718	+, *a5, r2*	+	*a2*	+, *n*	*a10, a20*	+	*a8, a22*
1741	~	~	*a2*	+	*a10*	+	+
1391	*r12*	+	*a2, a6?*	+	*a8*	+	*a8*
386	*r4*	*a4*	*a2, a6*	+	~	+	~
387	*r4*	*a4*	*a2*	+, *a5*	*a8*	+	*a8*
1352	~	~	*a2*	+	*a8, a20*	+	*a8, a22*
1353	*r4*	+, *a5*	+, *n*	+	*a8, a20*	+	*a8, a22*
1354	*r4*	+, *a5*	+, *a2, n*	+	*a8, a20*	+	*a8*
1347	*r4*	+, *a5*	+, *a2*	+	*a8, a20*	+, *a2*	*a8, a22*
1775C	*r2, r4*	+, *a5*	+, *a2*	+, *a2*	*a8*	*a2*	*a8*
1361	*r4*	+, *a5*	*a2*	*a2*	*a8*	+, *a2*	*a8*
1775F	*r2, r4*	+, *a5*	+, *r2*	*a2*	*a8*	*a2*	*a8, a24*
1362	*r2, r4*	+, *a5*	*a2*	*a2*	*a8*	*a2*	*a8, a24*
1780	*r2*	+, *a5*	*a2*	*a2*	*a8*	*a2*	*a8, a22*
1779	*r2, r4*	*a5*	+, *a2*	*a2*	*a8*	*a2*	*a8, a22*
1778	*r2, r4*	+, *a5*	+, *a2*	+, *a2*	*a8*	*a2*	*a8, a22*
1777	*r2, r4*	+, *a5*	+, *a2*	*a2*	*a8*	*a2*	*a8, a24*
1393	*r2, r4*	+, *a5*	*a2, a5*	*a2*	*a8*	*a2*	*a8, a24*
1366	*r2, r4*	*a5*	+, *a2*	+	*a8*	+	*a8*
1298	*r2*	*a5*	~	+, *a2*	*a8*	*a2*	*a24*
1761	*r2*	*a5*	*a2*	+	*a8*	*a2*	*a22*
1559	*r2*	*a5*	*a2*	*a2*	*a8*	*a2*	*a22*
94	*r2*	*a5*	*a2*	+	*a4, a20*	+	*a8, a22*
1764	*r2, n*	*a5*	*a2*	+, *a4*	*a8*	*a2*	*a22*
1772	*r2, n*	*a5*	*a2, a4*	+, *a4*	*a8*	+, *a2*	*a22*
1558	*r2*	*a5*	*a2*	*a2*	*a8*	*a2*	*a22*
1295	*r2*	*a5*	~	~	*a8*	+	*a8*

tected in accessions of *L. hirsutum*

x-1	*Prx-2*	*Prx-3*	*Prx-3b*	*Prx-4*	*Prx-7a*	*Prx-7*	% *polymorphic loci*
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*C*	+	+	0
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*D*	+	+	0
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*A, B*	+	+	8
1, r2	+	+	*r5, r7, n*	1 allele	+	+	17
1	+	+	*r5, n*	*A, B*	+	+	17
1, r2	+	+	*r3*	2 alleles	+	+	33
1, r2	+	+	*r5*	2 alleles	+, *a2*	+	17
1, r2	+	+	*r5*	+, *B*	+, *a2*	+	50
1, r2	+	+, *a3*	*r5, n*	+, *A, B*	+, *a2*	+	72
1	+	+, *a3*	*r5, n*	+, *A, B*	+, *a2*	+	57
1	+	+, *a5*	*r5, n*	2 alleles	+	+	25
1, r2	+	+, *a5*	*n*	*A*	+	*1*	21
1	+	+	*n*	*A*	+	+	8
1	+	+	*n*	+, *B*	+	+	14
1	+	+	*r3*	*A, B*	+	+	17
1	+, *r2*	+	*n, r3*	*B, C, D*	+	+	50
1, r2	+, *r2*	+	*n*	+, *A*	+, *n*	+	50
1, r2	+, *r2*	+	*n, r3*	*B, C, D*	+, *n*	+	72
1	+	+	*n*	*A*	+	+	29
1	+	+, *a5*	*n*	*B*	+	+	21
1	+	+	*n*	*A*	+	+	29
1	+, *r2*	+	*n*	*B*	+, *n*	+	36
1	+, *r2*	+	*n*	*B*	+	+	21
1	+	+	*n*	*B*	+	+	21
1	+, *r2*	+	*n*	*B*	+	+	43
1	+	+	*n*	*A*	+	+	29
1	+	+	*n*	*B*	+	+	29
, r2	+	+	*r5, n*	*C*	+, *n*	+	29
1	+	+	*r5, n*	+, *A, C*	+, *n*	+	29
1	+	+	*n*	*A*	+	+	0
1	+	+	*n*	*C*	+	+	0
1	+	+	*r5*	*C*	+	+	14
1	+	+	*r5, r7*	*A*	+	+	21
1	+	+	*n*	*A*	+, *a2*	+	36
1	+	+	*n*	*C*	+, *n*	+	7
1	+	+	*r5*	*C*	+	+	0

Table 2 (continued)

Access. No.	*Aps-1*	*Aps-2*	*Est-1*	*Got-1*	*Got-2*	*Got-3*	*Got-4*
1753	*n*	*a5*	*a2*	+, *a2*	*a8*	+, *a2*	*a8*
1560	*r2, n*	*a5*	*a2*	*a2*	*a8*	+	*a8*
1745	*r5*	*a5*	*a2*	*a4*	*a8*	+	*a8*
1696	*r2*	*a5*	*a2*	*a2*	*a8*	+	*a4*
1695	*r2*	*a5*	*a2*	*a2*	*a8*	+	*a4*
1681	*r2*	*a5*	*a2*	*a2*	*a8*	+	*a4*
1691	*r2*	*a5*	*a2*	*a2*	*a8*	+	*a4*
1730	~	~	*a2*	*a4*	*a8*	+	+
1731	~	~	*a2*	*a4*	*a8*	+	*a8*
1721	*r2*	*a5*	*a2*	+, *a2*	*a8*	+	*a8*

mations of allele frequencies. Accordingly, the situation at each locus is indicated by the number of kinds of alleles. The minimum proportion of allele frequency for polymorphy was set at 5%. A single value, per cent of loci that were polymorphic, was derived for each accession. Despite the crudeness of this criterion, highly significant trends in the data were thereby detected. Further, the higher the polymorphy, generally the higher the proportion of progeny plants that were heterozygous at the polymorphic loci.

The most dramatic feature of the results is the contrast between the zero or near-zero levels of polymorphy at the north and south margins of the distribution and the much higher levels in the more central regions. An irregular cline can be detected from the very low levels in the south toward higher levels in the central region, until maximum values are reached in the Piura-La Libertad-Cajamarca area, whence the percentages decline rapidly northward. These aspects of variability are evident in the graphical summary of results in Fig. 2. The results correlate remarkably with the geographic distribution of genetic variability observed in *L. pimpinellifolium* (Rick & al. 1977).

The next noteworthy item is the relationship between this geographic distribution of genetic variation and compatibility reactions: the regions of lowest variability are associated with SC, those of higher variability, with SI (Figs. 2, 3). Within the restrictions of the number of accessions available for testing, the boundary between the northern SC region and the central SI area corresponds exactly with the frontier between Ecuador and Perú. To the south, SC has been found in only the farthest drainages—Río Cañete, Río San Juan, and Río Pisco. The only exceptions are found in single accessions (LA94, 1295) each in the

ble 2 (continued)

rx-1	*Prx-2*	*Prx-3*	*Prx-3b*	*Prx-4*	*Prx-7a*	*Prx-7*	% *polymorphic loci*
	+	+	*n*	*B*	+	+	14
	+	+	*r5*	*A*	+	+	7
	+	+	*n*	*A*	+	+	0
	+	+	*n*	*F*	+	+	0
	+	+	*n*	*F*	+	+	0
	+	+	*n*	*F*	+	+	0
	+	+	*n*	*F*	+	+	0
	+	+	*r5*	*E*	+	+	0
	+	+	*r5*	*E*	+	+	0
	+	+	*r5*	*B*	+	+	7

Rímac and Chillón valleys, the next main drainages north of Río Cañete.

Other departures from this association of genetic variability and compatibility reaction are seen in three accessions—LA 1295, 1559, and 1761—which are SI yet displayed nil polymorphy. Whether these represent truly exceptional situations or sampling errors is not certain, but it is noteworthy that, as observed above, SC has been detected in these regions, which are otherwise completely SI. Furthermore, the general level of variability in these two valleys (Río Chillón and Río Rímac) is lower than that of other parts of the SI territory.

Pertinent to these considerations is the fact that, contrary to our results, both SC and SI were detected by MARTIN (1963) within the same populations of LA 387 (Santa Apolonia) and of PI 127, 827 collected at the Inca Baths near Cajamarca 19 years prior to our collection (LA 386) at the same site. The existence of SC plants in these populations might account for the fact that we measured considerably lower levels of polymorphy in them than in other accessions from NW Perú. It should also be noted that, in contrast to all of the more southerly distribution of *L. hirsutum*, these sites in the vicinity of Cajamarca lie east of the continental divide and might therefore represent another marginal, pioneering enclave.

Whatever the nature of these seeming exceptions, the difference in genetic variability between SC and SI accessions is impressive: the mean polymorphy for all SC populations is 1.5% whilst that for SI is 21.4%—a fourteen-fold difference. The statistical significance of this difference is beyond question because the two distributions scarcely overlap. The difference lies in the expected direction, but seems to

exceed the expected magnitude. Although the floral display of SC accessions is generally less than that of the SI group, flowers of the former nevertheless attract pollinating bees in considerable numbers according to our observations. Thus, we have witnessed visitations of bee spp. that were unmistakably gathering pollen from flowers of LA 1253 and 1264. SC therefore could hardly seem entirely accountable

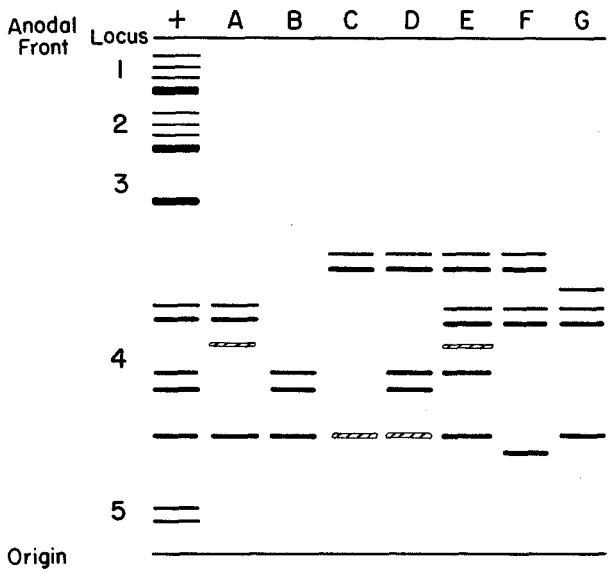
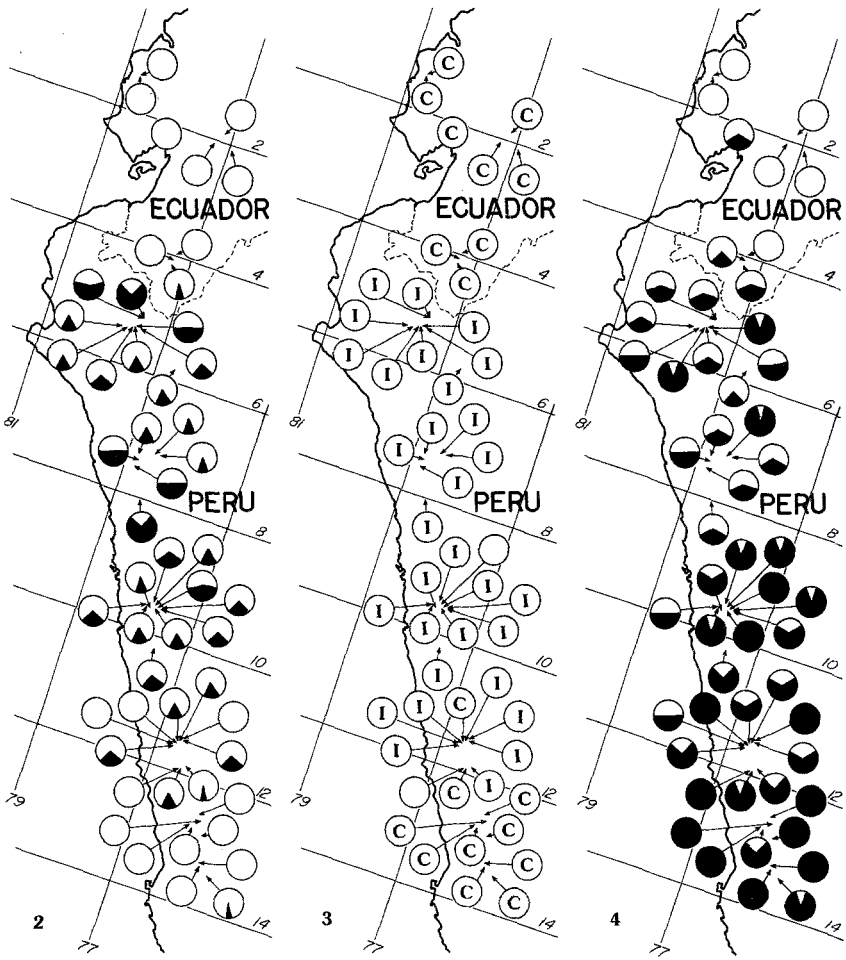


Fig. 1. Phenotypes of alleles of *Prx-4* in *L. hirsutum*. Mean distance from origin to anodal front is 10.8–11.0 cm. Full array of anodal peroxidase bands is illustrated only for *Prx-4*$^+$ at left

for the near-zero variability of the SC accessions. A similar relationship between compatibility types and extent of variability has been observed by Levin (1978) between *Phlox* spp. and by Solbrig & Rollins (1977) between *Leavenworthia* spp.; however, Jain (1976) and others, pointing out various exceptions, caution against expecting correlations between rates of outcrossing and degree of genetic variability to be universal. In such studies it has seldom been possible to make extensive comparisons *within* species as permitted by *L. hirsutum*. The hazards of drawing conclusions in comparisons *between* species with their widely different adaptations, degrees of polyploidy, rates of reproduction, and other factors are obvious.

The data suggest that in the migration of *L. hirsutum* from the central region, advantage of fixing one or a few highly successful genotypes or the failure to obtain the necessary population size or



Figs. 2 4. Geographic relationships for genetic variability, self-incompatibility, and alleles present at several loci. Circles represent populations sampled.—Fig. 2. Proportion of tested loci that were polymorphic (percent polymorphy) represented by the black portion of each circle.—Fig. 3. Compatibility relations. C = self-compatible; I = self-incompatible.—Fig. 4. Total scores of five loci differentiating northern and southern elements of *L. hirsutum*. Northern alleles represented by white; southern alleles, by black. See text for further information

pollen vectors might have resulted in substitution of SC. Consideration of the problems in the evolution of compatibility types by LEWIS & CROWE (1958), DE NETTANCOURT (1977) and others leads to the conclusion that the evolution of SC from SI is far simpler than the reverse. For this and other reasons SI is generally considered ancestral to SC.

Hypotheses based on a common origin for the two groups of SC accessions are negated by the genetic differences observed between the two, as delineated below.

Regional Trends; Morphological Characters

The northern and southern SC groups differ in the alleles present at five of the fourteen investigated enzyme loci. Thus at *Aps-2* the + and *a2* alleles are fixed respectively; for *Got-1*, + and *a2*; for *Got-2*, *a20* and

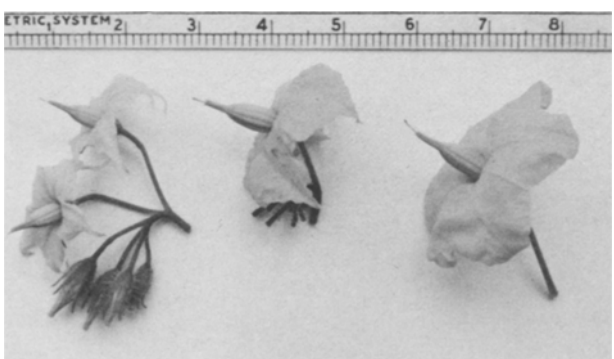


Fig. 5. Representative flowers of three accessions of *L. hirsutum*. Left: LA407 (Guayaquil, self-compatible). Center: LA 1721 (Tiarapo Viejo, self-compatible). Right: LA1772 (Canta, self-incompatible). Note differences in corolla size

a8; for *Prx-4*, the *A* allele vs the others; and for *Prx-3b*, the *r4* band was present in some southern accessions but was never detected in the northern group. This substitution of alleles at 36% of the loci constitutes a remarkable degree of genetic differentiation and a formidable argument against a close phylogenetic relationship between the two groups. Further, the electrophoretic data are consistent with observations on divergence in leaf size, flower size and color, and other morphological characters. The differentiation of northern and southern biotypes is summarized in Fig. 4 for these enzyme loci omitting *Prx-3b*. All characters are given equal weight, the northern alleles signified by white, the southern alleles by black.

Other interesting features are the observed morphological differences that tend to be associated with SC. Corolla limb diameter is generally smaller than that of the SI populations, the difference being greater in the northern SC group (Fig. 5). These changes in the SC races evidently evolved as a result of the lack of selection pressure for attractivity to pollinating bees, so essential to the reproduction of SI

biotypes. A strange but consistent difference seen in both northern and southern SC groups is the tendency toward thinner stems, more diminutive plant parts, and greater basal branching than in the SI accessions as a whole. Presumably the fixation of these characters also reflects the action of natural selection since they are fixed in both ends of the distribution, but the basis for such selection is not clear at this time.

As previously mentioned, additional loci of *Est* and *Pgm* were tested for certain but not all accessions. These additional data can be summarized briefly as follows. Six SC accessions were tested for two *Pgm* and ten for the five additional *Est* loci; all accessions proved to be homomorphic for these loci. Amongst the SI accessions, 15 were tested for *Pgm* and 10 for the additional *Est* genes; five were polymorphic for at least one *Pgm* and eight were polymorphic at one or more *Est* loci. These results are therefore concordant with the completely tested loci to the extent that no variability was found in any SC accession in contrast with the extensive fluctuations seen in the SI material. It is also of interest to note that variation at the additional loci was encountered for a SI accession (LA 1761) that proved homotypic in the tests for other electrophoretic loci. This information suggests that the uniformity registered for the 14 standard genes might have been an artifact resulting from small sample size and/or inbreeding during accession seed increases. In summary, these additional data lend further support to the previously observed marked differences in genetic variability between the SC and SI groups.

As to gross morphology, we have been impressed by the generally low level of variation within populations, whether in the wild or test progenies in culture. In contrast, the accessions, particularly those from different drainages, often differ from each other in such features as size of vegetative parts, hairiness, anthocyanin pigmentation, and various features of the calyx, fruit size and pattern of coloration. Marked gross morphological variability has been observed *within* accessions from the Huancabamba region (the northernmost Peruvian collections), where electrophoretic polymorphy also reaches a maximum. This region is also unique for heteromorphy at the hair control locus (*h*) (Table 1).

Insofar as comparisons are permitted, the picture of morphological variability agrees with that for zymotypes. The two sets of data are concordant in that: 1) races from different valleys are considerably differentiated from each other; 2) populations that are monomorphic in zymotype tend to be uniform in gross morphology; and 3) populations with greatest zymotypic polymorphy tend to be variable in gross morphology. Previous instances of such agreement were found in *L. cheesmanii* (Rick & Fobes 1975a), *L. pimpinellifolium* (Rick & al. 1977),

and the sibling species *L. chmielewskii* and *L. parviflorum* (Rick & al. 1976). These accrued examples lend abundant support to the use of isozymes as indices of genetic variation in the tomato species.

General Considerations

The preceding observations support the following hypothesis for evolution of *L. hirsutum*. Assuming that SC forms evolved from SI, the central populations would be ancestral to those in the northern and southern margins of the distribution. Within the central SI group, the northern populations (Dept. Piura, Cajamarca, La Libertad) exhibit the greatest variation. We therefore suggest that they also represent the oldest living biotypes of *L. hirsutum*, from which the others were derived. As the species migrated northward and southward through its elongated distribution, SI was replaced by SC possibly as a result of selection for greater uniformity and for fewer and more weedy genotypes and/or of founder events in which SC might have been essential for the survival in diminished populations, in conformity with Baker's (1955) law.

It is noteworthy that the patterns of variability and suggested evolution of mating systems in relation to geographic distribution for *L. hirsutum* conform with remarkable exactness with those proposed for *L. pimpinellifolium* (Rick & al. 1977). Although the latter species is entirely SC, outcrossing in the marginal populations at zero or near zero levels grades to a maximum in the central region. These extraordinary parallels are even matched by similar changes in flower size. The two systems must have evolved independently since the two species are not closely related. It is also significant that the evidence points to the same general region as the center of origin of both species. This area is also unique floristically for the presence of an unusually large number of endemic species, also because it is transitional between the moist tropics of the Ecuadorean habitats to the north and the temperate deserts to the south.

Summary

Variation was measured for allozymic banding at fourteen genetic loci in acid phosphatase, esterase, glutamate oxaloacetate transaminase, peroxidase, and phosphoglucomutase and for morphological characters in progenies of wild populations of *Lycopersicon hirsutum*. Flower size was measured and reactions to self-pollination tested in these progenies grown at Davis, California. In the elongate distribution of this species oriented NNW-SSW, allozymic polymorphy varied from zero in the northernmost and southernmost populations to values as

high as 72% in northwestern Perú. Populations at the two extremities differed in alleles fixed at five (34%) of the total tested genes. Similar variation was observed in corolla size and color, leaf dimensions, epidermal hairs, and plant habit. Self-compatibility was detected in these marginal populations and two more centrally located accessions, self-incompatibility in the remainder of the range. The degree of variation in all observed characters was vastly greater in the latter than in the former group, the difference doubtlessly owing in part to the difference in breeding systems. Self-compatibility is also associated with smaller corolla dimensions than self-incompatibility, the difference being greater in the North. These patterns of variability and fixation of alleles coincide very closely to those previously discovered in *L. pimpinellifolium*. The hypothesis of evolution in *L. hirsutum*, as also proposed for the other species, that is most compatible with the data assumes that the more uniform self-compatible races at the extremities of the distribution were derived progressively from the self-incompatible biotypes in the central region of high variability.

Research supported in part by NSF Grant DEB 77-02248. We gratefully acknowledge the indispensable assistance of the following colleagues and assistants. Various accessions of *L. hirsutum* were collected and contributed by Ing. David Baumann, Dr. Raymond Clark, Ing. Tomás Guerrero, Dr. Miguel Holle, Ing. Daniel Nakama, Dr. Carlos Ochoa, Ing. Eduardo Vallejos, and Dr. Harold Winters. Field and greenhouse operations were directed by Paul Bosland, assisted by Craig Gianinni, Dora Hunt, Herculano Medina, and Randy Schuster. Dora Hunt assisted in editing the manuscript and Moira Tanaka did the cartography.

References

Baker, H. G., 1955: Self-compatibility and establishment after long-distance dispersal. — Evolution **9**, 347—348.

Jain, S. K., 1976: Population structure and the effects of breeding system. In Frankel, O. H., Hawkes, J. G. (Eds.): Plant Genetic Resources: Today and Tomorrow. — London: Cambridge Univ. Press.

Levin, D. A., 1978: Genetic variation in annual phlox: self-compatible versus self-incompatible species. — Evolution **32**, 245—263.

Lewis, D., Crowe, L. K., 1958: Unilateral interspecific incompatibility in flowering plants. — Heredity **12**, 233—285.

Martin, F. W., 1963: Distribution and interrelationships of incompatibility barriers in the *Lycopersicon hirsutum* Humb. & Bonpl. complex. — Evolution **17**, 519—528.

McGuire, D. C., Rick, C. M., 1954: Self-incompatibility in species of *Lycopersicon* sect. *Eriopersicon* and hybrids with *L. esculentum*. — Hilgardia **23**, 101—124.

Muller, C. H., 1940: A revision of the genus *Lycopersicon*. — U.S. Dept. Agric. Misc. Publ. **382**.

Nettancourt, D. de, 1977: Self-incompatibility. — Berlin: Springer.
Rick, C. M., Fobes, J. F., 1975a: Allozymes of Galápagos tomatoes: polymorphism, geographic distribution, and affinities. — Evolution **23**, 443—457.
— — 1975b: Allozyme variation in the cultivated tomato and closely related species. — Bull. Torrey Bot. Club **102**, 376—384.
— — 1976: Peroxidase complex and concomitant anodal and cathodal variation in red-fruited tomato species. — Proceedings, National Academy of Science **73**, 900—904.
— — Holle, M., 1977: Genetic variation in *Lycopersicon pimpinellifolium*: evidence of evolutionary change in mating systems. — Pl. Syst. Evol. **127**, 139—170.
— Holle, M., Thorp, R. W., 1978: Rates of cross-pollination in *Lycopersicon pimpinellifolium*: impact of genetic variation in floral characters. — Pl. Syst. Evol. **129**, 31—44.
— Kesicki, E., Fobes, J. F., Holle, M., 1976: Genetic and biosystematic studies on two new sibling species of *Lycopersicon* from interandean, Perú. — Theor. Appl. Genetics **47**, 55—68.
Singh, R., 1976: Substrate-specific enzyme variation in natural populations of *Drosophila pseudoobscura*. — Genetics **82**, 507—526.
Solbrig, O., Rollins, R. C., 1977: The evolution of autogamy in species of the mustard genus *Leavenworthia*. — Evolution **31**, 265—281.
Stoner, A. K., 1970: Breeding for insect resistance in vegetables. — HortScience **5**, 76—79.

Addresses of the authors: Dr. Charles M. Rick and Mr. Steven D. Tanksley, Department of Vegetable Crops, University of California. Davis, California 95616, U.S.A.—Dr. Jon F. Fobes, Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, U.S.A.