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Alterations of the manifestations of hybrid breakdown in *Lycopersicon esculentum* *L. pennellii* F_2 populations containing *L. esculentum* versus *L. pennellii* cytoplasm

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Abstract Reproductive abnormalities reduced the percent stainable pollen, and fruit and seed set in interspecific F_2 populations derived from crosses of *Lycopersicon esculentum* and *L. pennellii* but were not observed in parental lines and interspecific F_1 populations. The degree to which these reproductive abnormalities were expressed in the interspecific F_2 populations was affected by cytoplasm. Reproduction was impeded in interspecific F_2 populations containing *L. esculentum* cytoplasm (F_2^{Le}) by reduction in pollen production, the lack of fruit set and a high proportion of parthenocarpic fruit among plants capable of fruit set. The F_2 populations containing *L. pennellii* cytoplasm (F_2^{Lp4}) showed a reduced frequency of reproductive abnormalities at all stages of reproductive development, resulting in higher values for percent stainable pollen, fruit and seed set and higher proportions of the F_2^{Lp4} populations being capable of setting fruit or seed than F_2^{Le} populations. The major barrier remaining in F_2^{Lp4} populations was reduced fruit set compared to parental lines. The barrier to fruit and seed set observed in the F_2^{Le} populations, and to a lesser extent in the F_2^{Lp4} populations, occurs around the time of fertilization or early embryonic development. The effect of *L. pennellii* cytoplasm on barriers in the F_2^{Lp4} populations is proposed to be due to an interaction between cytoplasmic and nuclear genes during fertilization of the F_1 plants to produce F_2 populations and may also affect subsequent generations.

Key words *Lycopersicon* · Interspecific cross · Hybrid breakdown · Fecundity · Nuclear-cytoplasmic interaction

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Introduction

Wild relatives of crop species are important sources of valuable traits that enable plant breeders to produce varieties that better fit agricultural requirements. Reproductive barriers restrict the flow of genes between species and thus are major obstacles to the use of wild species in breeding programs. These barriers can be detected at several developmental stages (Knox 1984; Ascher 1986), including pollen tube growth, fertilization and seed development, and they may result in a lack of fertilization, embryo abortion, poor embryo development, or decreased seed viability.

The interspecific barrier, hybrid breakdown, can affect the F_1, F_2, or later generations following an interspecific cross, resulting in plants that are non-vigorous, male and/or female sterile, or non-fecund (fail to produce viable seed) (Hadley and Openshaw 1980; Weeden and Robinson 1986; Haghighi and Ascher 1988). Reproductive abnormalities in F_2 populations caused by hybrid breakdown were reported in crosses between *Gossypium hirsutum* L. and *G. barbadense* L. (Stephens 1950), *Zauschneria cana* Greene and *Z. septentrionalis* Keck (Clausen et al. 1940), *Layia gaillardioides* DC. (Hook. et Arn.) and *L. hieracioides* DC. (Hook. et Arn.) (Clausen 1951), *Curcurbita maxima* Duchrsne and *C. ecuadorensis* Cutler and Whitaker (Cutler and Whitaker 1969; Weeden and Robinson 1986) and crosses between most of the species in *Lycopersicon* (MacArthur and Chiasson 1947; Rick and Lamm 1955; Rick et al. 1976; Mutschler and Liedl 1994). The presence of a complete genomic set of chromosomes from each of the parent species did not affect plant growth or fertility in these interspecific F_1s, which suggests that hybrid breakdown in these cases is not due to gross chromosomal differences between the species. Weakness and sterility in later generations appeared only after recombining the alleles from the two species.

Little is known about the mechanisms of reproductive barriers, such as hybrid breakdown. *Lycopersicon* is a good research model for work on reproductive barriers because all members of the genus have the same chro-

mosome number and chromosome structure (Rick 1991), and crossing behaviors among the different species in the genus have been well established (see summary in Mutschler and Liedl 1994).

We were interested in the reproductive barriers between *L. esculentum* and *L. pennellii* because multigenic traits of horticultural interest are found in the wild species, *L. pennellii*. Since self-compatible accessions of *L. pennellii* are available and all accessions of *L. esculentum* are self-compatible, self-incompatibility should not confound the effects of the interspecific reproductive barriers. Prior work with these two species identified hybrid breakdown in the F_2 generation. Interspecific hybridization produced a fertile hybrid, which gave rise to an F_2 population exhibiting severely depressed fecundity, regardless of the accessions used in the initial interspecific cross (C.M. Rick, personal communication).

Andersen (1963) reported creation of sterile plants by transferring the *L. pennellii* nuclear genome into the cytoplasm of *L. esculentum*. He also proposed that transfer of the *L. esculentum* nucleus into *L. pennellii* cytoplasm might create a cytoplasmic male sterile. Creation of cytoplasmic/nuclear male sterility by interspecific crosses has been reported in many crop plants (Hanson and Conde 1985; Kaul 1988). Inheritance of organelles in tomato is uniparental for *Lycopersicon* species (Smith 1989). Thus, in crosses between *L. esculentum* and *L. pennellii*, the progeny all carry the *L. esculentum* organelles when *L. esculentum* is the female parent. Comparison of the two F_1s generated between these species would be informative to determine whether there is an interaction between the cytoplasm and the nucleus affecting hybrid breakdown. However, the reciprocal cross (*L. pennellii*×*L. esculentum*) is not successful, due to an unrelated reproductive barrier, unilateral incongruity (Mutschler and Liedl 1994).

The unique isocytoplasmic line, consisting of an *L. esculentum* nucleus and *L. pennellii* organelles, was created from embryos rescued from a cross between *L. pennellii* and its interspecific F_1 (*L. esculentum*×*L. pennellii*) (Mutschler 1990). This line is fertile and fecund, rather than cytosterile, as suggested by Andersen (1963). In the process of developing the isocytoplasmic line, the reproductive behavior of intervening generations suggested that the *L. pennellii* cytoplasm reduced reproductive abnormalities in succeeding generations. The objectives of this study were to define hybrid breakdown in an F_2 population of *L. esculentum*×*L. pennellii* and ascertain the effect of the cytoplasm on hybrid breakdown in the F_1 and F_2 generations.

Materials and methods

Plant materials

The plant materials used included *L. esculentum* cv. New Yorker (NY), which is self-compatible (as are all *L. esculentum* cultivars), a self-compatible accession of *L. pennellii* PI 246502, LA716 (Lp) from Dr. C.M. Rick, University of California, Davis (Rick 1960, 1969), and the interspecific hybrid between these two lines (NY×Lp). This interspecific F_1 hybrid contains the *L. esculentum* cytoplasm, and so is referred to as F_1^{Le}, in which the superscript "Le" denotes the cytoplasm type. The F_2 seeds obtained from self-pollinations of a single F_1^{Le} plant were the basis of the F_2^{Le} populations.

The "isocytoplasmic" line contains the cytoplasm of Lp and the nuclear genome of NY (Mutschler 1990). The fourth true backcross generation in the creation of the isocytoplasmic line (NY^{Lp4}), which has the pedigree ((((((Lp×F_1^{Le})×F_1^{Le})×F_1^{Le})×NY)×NY)×NY)×NY, was pollinated with Lp pollen to give the interspecific hybrid F_1^{Lp4}. Seed collected from self-pollination of a single F_1^{Lp4} plant was the basis of the F_2^{Lp4} populations. RFLP analysis with average spacing between markers of approximately 15.5 cM through the entire nuclear genome later indicated that the genome of the NY^{Lp4} plant used in the creation of the F_1^{Lp4} and the F_2^{Lp4} populations was homozygous *L. esculentum* except for a region on chromosome 1 between the markers CT233 and TG71 from the map of Tanksley et al. (1992) that was heterozygous. Further RFLP analysis of the F_1^{Lp4} and the F_2^{Lp4} populations showed that their genomes were homozygous *L. pennellii* for the same region on chromosome 1.

Two F_2^{Le} populations (I, III) and two F_2^{Lp4} populations (II, IV) were studied (Table 1). Populations I and II contained more individuals than populations III and IV; however, populations III and IV have the advantage of being grown simultaneously in the same environment. The parental lines NY, NY^{Lp4} and Lp, as well as F_1^{Le} and F_1^{Lp4}, were grown at the same time with each F_2 population for use as controls. All plants were grown under greenhouse conditions in soilless media (a modification of Cornell mix (Boodley and Sheldrake 1982)) supplemented with Osmocote and fertilized weekly with Peters 9–14–15. Natural daylength conditions were supplemented to 12 h (0600–1800 h) by high-intensity discharge lights. Standard fungicide and insecticide practices were followed.

Pollen stainability

Three flowers from each plant were collected in the morning, fixed for 24 h in Carnoy's fixative (3 absolute ethanol: 2 chloroform: 1 glacial acetic acid), and subsequently stored in 70% ethanol at 4°C. The anthers of each flower were macerated with a drop of Alexander's stain (Alexander 1969) and examined microscopically at 80 to 100× to determine the percentage of stainable pollen. Stainable pollen grains (fertile) were plump and purple, due to stained cytoplasm, and surrounded by a ring of green, due to the stained cell wall. In contrast, unstained pollen grains (aborted) were shriveled and were only stained green because they lacked cytoplasm. The pollen stainability of each plant was calculated by averaging the percentages of the three flowers. Additional samples were counted to confirm the data from plants showing either a large variation in the percent pollen stainability among the flowers, very few pollen grains, or no pollen observed in the three flowers collected. Average percent pollen stainability data were transformed by the arc sine function to approach normality for statistical tests. The differences between population means in percent pollen stainability were tested by *t*-tests without pooling the variances, using statistical software (MINITAB 1991). Anthers of the plants showing no pollen were stained with Alexander's stain and examined under a light microscope. All of the flowers showing no pollen production and no evidence of microsporocyte development from the microspore mother cells were considered to have a premeiotic block to pollen production.

In vitro and in vivo pollen germination

The normal appearance of plump stainable pollen does not prove pollen viability. Therefore, pollen was tested by using the following in vitro and in vivo pollen germination tests to determine whether pollen stainability accurately reflects pollen viability, and

Table 1 Comparison of the four F_2 populations for reproductive potential, pollen stainability, fruit set, seed set and germinable seed

Characteristic	Population I (F_2^{Le})		Population II (F_2^{Lp4})		Population III (F_2^{Le})		Population IV (F_2^{Lp4})	
	n	%	*n*	%	*n*	%	*n*	%
Population size	146		74		53		59	
Non-flowering	25	17.1	1	1.4	5	9.4	0	0.0
Premeiotic block	4	2.7	0	0.0	2	3.8	0	0.0
Pollen stainability	117		73		46		59	
mean[a] (SEM)[b]		50.0 (2.23)	74.4 (2.47)		37.8 (4.25)		62.1 (2.58)	
median[a]		53.8		81.4		36.8		63.2
range[a]		0.0–90.6		0.0–97.5		0.5–86.7		6.7–94.7
Fruit set	23	19.7[a]	54	74.0[a]	21	45.7[a]	33	55.9[a]
Fruit parthenocarpic	23	100.0[c]	3	5.6[c]	9	42.9[c]	1	3.0[c]
Fruit contains seed	0	0.0[c]	51	94.4[c]	12	57.1[c]	32	97.0[c]
Mean seed per fruit			4.2	10.9		4.2		10.9
Set germinable seed	0	0.0	48	94.1[d]	10	83.3[d]	30	93.8[d]
Fecund plant	0	0.0, 0.0[e]	48	65.8, 64.9[e]	10	21.7, 18.9[e]	30	50.8, 50.8[e]

[a] Percentage based on the portion of the F_2 population which produced pollen
[b] SEM: standard error of the mean
[c] Percentage based on the portion of the F_2 population which set fruit
[d] Percentage based on the portion of the F_2 population which set seed
[e] Percentages based on the portion of the F_2 population which produced pollen, and on the entire population, respectively

thus whether the crossing barrier resulted from a lack of viable pollen. Eight plants from population III and seven plants from population IV having highest pollen stainability within the populations, but differing for fruit and/or seed production, were chosen for the in vitro and in vivo pollen germination tests. Due to fluctuations in flowering dates and the need for replication, tests were repeated several times.

For in vitro pollen germination tests, pollen was collected in the morning by vibrating freshly opened flowers over a microfuge tube. The pollen was then suspended at a concentration of 2.5×10^5 pollen grains/ml of pollen germination solution [10% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)\cdot4H_2O$, 200 ppm $MgSO_4\cdot7H_2O$ and 100 ppm KNO_3 (pH 6.5) (Brewbaker and Kwack 1963)]. Each pollen suspension was replicated three times, with 100 µl of the pollen suspension distributed into three wells of a 96-well microtiter plate, which was held for 4 h on an orbital shaker at very low speed at room temperature. Afterwards, a drop of the pollen suspension from each well was stained with a drop of Alexander's stain, and examined microscopically. Germinated pollen grains were characterized by pollen tubes at least as long as the diameter of the pollen grains. The germinable pollen was expressed as the percentage of total stainable pollen grains; the in vitro pollen germination percentage for each plant was calculated by averaging the three replicates. Pollen from NY, Lp, NY^{Lp4}, F_1^{Le} and F_1^{Lp4} plants were used as controls to standardize the procedure.

For in vivo pollen germination tests, fresh shoots with flowers and buds were taken from the greenhouse in the morning and placed in water pics containing 10% sucrose and 200 ppm 8-hydroxyquinoline (Flaschenriem 1974). The buds were emasculated and subsequently pollinated with pollen from open flowers on the same shoot. These shoots were kept in a growth chamber maintained at 22°C. After 48 h, the pollinated flowers were placed in FPA fixative (5% formalin, 5% propionic acid, and 90% ethanol) at 4°C until examination. The sepals, petals, and any remaining anther cone tissue were dissected away from the pistil before it was softened in 8 N NaOH overnight. The NaOH was then removed and the pistils were rinsed three times with double-distilled water. The pistils were stained with aniline blue (0.1% aniline blue in 0.1 M K_3PO_4) for 4 h in the dark, placed in a drop of glycerine, squashed on a slide, and examined by fluorescent light microscopy (Zeiss standard microscope no. 2 filter) (Kho and Baer 1968).

Seed production

At least five flowers per F_2 plant were self-pollinated manually to produce F_3 seed. On those F_2 plants not initially setting fruit, additional manual pollinations were made with both self and Lp pollen. Self-pollinations were also made continuously on all plants, including the controls, using floral vibration during the 4-month period after plants began flowering. The fruit from each plant was collected at maturity and seed was extracted by soaking the seed and placental tissue for 20 min in 18% HCl to remove the placental tissue from the seed. Numbers of total fruit and total seed harvested were collected for each plant and converted to average seed per fruit (total seed harvested/total fruit harvested).

Seed germination test

Seed germination tests were performed on seed for each F_2 plant that had seed set and for the controls, NY, Lp, NY^{Lp4}, F_1^{Le} and F_1^{Lp4}. Up to 50 seeds per plant were tested by placing the seed on moist blotting paper in a plastic box, and holding the box in a dark growth chamber maintained at 30°C (Association of Official Seed Analysts 1988). The total number of seedlings was recorded on the 5th and 14th days after the start of the test. The few seeds that developed abnormal roots or cotyledons and stopped growing were recorded as abnormal/stunted seedlings and were not scored as germinable seed. The percent seed germination of each plant was expressed as the percentage germinable seed of the total seed tested.

A second seed germination test was done with the seed that failed to germinate in the first germination test to determine whether the seeds were dormant (Rick and Hunt 1961). The ungerminated seeds were soaked in 2.7% sodium hypochlorite for 30 min, thoroughly rinsed in tap water, dried, then retested for germination using the procedure described above.

Results

Reproductive parameters

All plants from the control populations (NY, NY^{Lp4}, Lp, F_1^{Le} and F_1^{Lp4}) flowered and produced pollen normally,

but some of the plants in the F_2 populations showed reproductive abnormalities not observed in the control populations. In the F_2^{Le} populations I and III, 19.8% and 13.2%, respectively, of the plants either failed to flower or to produce pollen due to premeiotic failure (i.e. preanthesis bud abortion or premeiotic blocks in microsporogenesis) (Table 1). However, failure to flower or produce pollen was much rarer (1.4%) and not observed, respectively, in the F_2^{Lp4} populations II and IV (Table 1).

Pollen viability, as measured by percent pollen stainability, was reduced in the F_2^{Le} and F_2^{Lp4} populations compared to the control lines. Estimated population mean values (and standard errors of the means) of percent pollen stainability for the control lines NY, NY^{Lp4}, Lp, F_1^{Le} and F_1^{Lp4} were high, at 95.5% (1.05), 97.3% (0.51), 87.6% (0.92), 79.2% (1.10) and 86.0% (0.84), respectively. All of the F_2 populations had lower mean percent pollen stainabilities with much higher standard errors than the controls (Table 1). Considering the similar small variances of the parent controls, the higher variances of the F_2 populations are likely to be the result of segregation for gene(s) affecting pollen production.

There were also significant differences in percent pollen stainability between the F_2^{Le} and F_2^{Lp4} populations. Both of the F_2^{Lp4} populations had higher percent pollen stainabilities than the F_2^{Le} populations (Table 1). Significant differences in the mean values of pollen stainability were observed between populations I and II (t-value=7.83, df=148, $P<0.001$) and between populations III and IV (t-value=4.85, df=81, $P<0.001$).

Fruit and seed set were also reduced in the F_2 populations, with both of the F_2^{Le} populations being more severely affected than either of the F_2^{Lp4} populations. All the plants of parental lines NY, NY^{Lp4}, Lp, F_1^{Le} and F_1^{Lp4} set fruit and seed successfully after being self-pollinated by hand or by floral vibration, although Lp plants set less fruit than the other parental lines after floral vibration, and also produced some parthenocarpic fruit. All four F_2 populations set less fruit and seed than the parental lines and the two F_1 populations, even after intensive manual self-pollination (Table 1). The percentage of plants producing only parthenocarpic fruit was much larger in the F_2^{Le} populations (I and III) than the F_2^{Lp4} populations (II and IV). However, no correlation was observed between percent pollen stainability and fruit set in either F_2^{Le} population. Percent pollen stainability of population I and III plants that set only parthenocarpic fruit ranged from 6% to 87%.

The number of seeds produced in seed-bearing fruit was also reduced in both the F_2^{Le} and F_2^{Lp4} populations compared to the controls. The average seed number per seed-bearing fruit from manual self-pollinations of the control populations was 44.6 for NY plants, 41.9 for NY^{Lp4} plants, and 14.3 for Lp plants. The average seed number per seed-bearing fruit was higher for F_1^{Lp4} plants (35.4) than for F_1^{Le} plants (26.6). The average seed number per seed-bearing fruit was much lower in fecund plants in the F_2^{Le} and F_2^{Lp4} than in the parent lines and the F_1^{Le} and F_1^{Lp4}, but again the average seed number per seed-bearing fruit was higher for seed-producing plants with *L. pennellii* cytoplasm (F_2^{Lp4}) than for plants with *L. esculentum* cytoplasm (F_2^{Le}) (Table 1). Reduced seed set in both the F_2^{Lp4} and F_2^{Le} was not caused by abnormalities in the development or function of the female structures, since all of the non-fecund F_2^{Lp4} and F_2^{Le} plants tested were capable of fruit and seed set when pollinated with Lp pollen.

Since a plant cannot be considered fecund unless it is demonstrated that the seeds it produces are viable, germination was tested to determine seed viability of the controls, remnant F_2^{Le} and F_2^{Lp4} seed, and F_3 seed lots generated by selfing F_2 plants in populations II (F_2^{Lp4}), III (F_2^{Le}), and IV (F_2^{Lp4}). Variation in percent germination existed among the control seed. The percent germination for Lp seeds was only 18% after 14 days. The very low percent germination of the seed of this wild species may be due to the presence of some non-viable immature seed in the seed bulk used; therefore, this population was eliminated from further comparisons among the generations. Self seed from the NY plants reached 100% germination by the 14th day, while percent seed germination at 14 days was slightly lower for seed of NY^{Lp4} (86.0%), F_1^{Le} (93.8%) and F_1^{Lp4} (83.3%). However, since similar differences had not been observed between other seed lots of NY and NY^{Lp4} (E.D. Cobb, personal communication), no consistent effect on seed germination was ascribed to the cytoplasmic and nuclear differences between the NY and NY^{Lp4} or, by extension, between the F_1^{Le} and F_1^{Lp4}. Percent germinability was decreased in remnant F_2^{Lp4} and F_2^{Le} seed (both 80.0%) to a level similar to that of F_1^{Lp4}.

Percentage germinability was decreased in F_3^{Le} and F_3^{Lp4} seed lots. Only five of the ten F_3^{Le} seed lots obtained from seed-producing F_2^{Le} plants in population III had greater than 50% germination, and the average number of F_3^{Le} seeds available for testing was only 13 per plant. However, 73% (35 of the 48 plants tested) and 87% (26 of the 30 plants tested) of the F_3^{Lp4} seed lots obtained from F_2^{Lp4} plants in populations II and IV, respectively, had seed germination higher than 50%, and the average number of F_3^{Lp4} seeds tested was 34 per plant. No correlation between the number of seeds produced and the percentage of seeds that germinated was found within any of the populations. The mean percent germination of F_3^{Lp4} seed (59.0 and 62.2% from populations II and IV, respectively) was distinctly higher than that of F_3^{Le} seed (45.7 from population III), repeating the general pattern of reduced expression of reproductive abnormalities in the F_2^{Lp4} compared to that in the F_2^{Le} (Table 1). A similar small proportion of the F_3 seed lots from the F_2 populations (one of the 12 obtained from population III F_2^{Le} plants, three of the 51 population II and two of the 32 population IV F_2^{Lp4} plants) produced only non-germinable F_3 seeds. Seed dormancy was not indicated, since the sodium hypochlorite treatment of the non-germinated seed did not result in any improvement in seed germination.

The type of abnormalities observed affecting fecundity were consistent between the F_2^{Le} and F_2^{Lp4} populations,

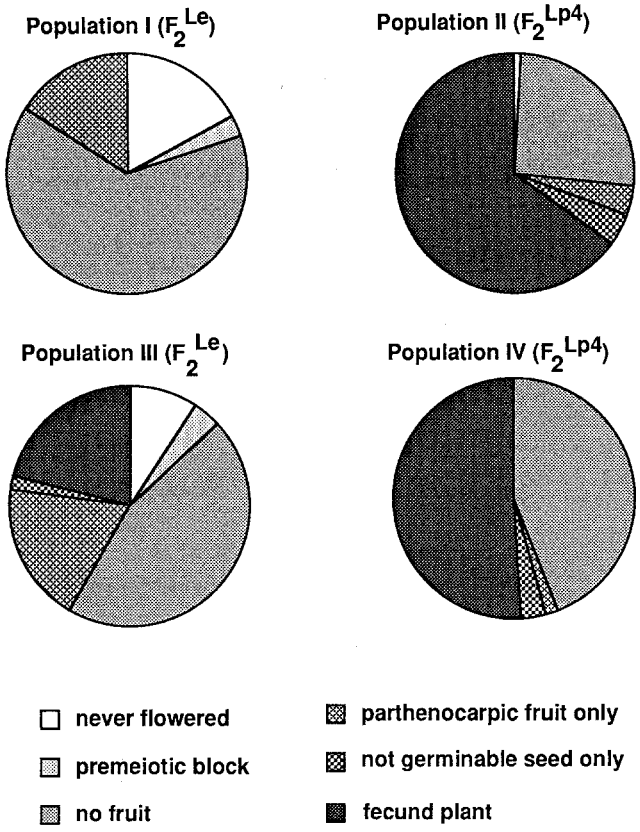

Fig. 1 Comparison of reproductive parameters measured for F_2^{Le} populations (populations I and III) and F_2^{Lp4} populations (populations II and IV)

with a greater proportion of F_2^{Le} plants producing abnormal or stunted F_3 seedlings than F_2^{Lp4} plants. Of the F_3 seed lots tested, 33% (four of the 12 obtained from population III) produced abnormal or stunted F_3 seedlings, while 15.7% and 21.9% of the F_3^{Lp4} seed lots (eight of the 51 obtained from population II and seven of the 32 obtained from population IV F_2^{Lp4} plants, respectively) produced abnormal or stunted F_3 seedlings. However, the number of abnormal or stunted F_3 seedlings produced by any F_2 individual was very small.

Overall, fewer F_3 plants were generated from a smaller proportion of individuals in the F_2^{Le} populations than in the F_2^{Lp4} populations. No fecund plants were obtained from the F_2^{Le} population I. Of the 12 F_2^{Le} seed-bearing plants in population III, ten fecund plants gave 88 F_3^{Le} seedlings, of which 57 were derived from only two relatively productive F_2^{Le} plants. In contrast, of the 83 seed-bearing plants in the combined F_2^{Lp4} populations II and IV, 78 were fecund; 24 of these produced more than 100 seeds each.

In summary, some plants within each F_2 population exhibited one or more form of blocked reproductive development. These restrictions to reproduction were less frequent in F_2^{Lp4} populations than in F_2^{Le} populations, resulting in higher values for mean pollen stainability, fruit set, seed set, and seed germination and higher proportions of plants capable of setting fruit or seed in the F_2^{Lp4} populations (Fig. 1). The cumulative effect of these restrictions is that the proportion of an F_2 population that is fecund (capable of producing germinable seed) was lower in the F_2^{Le} populations (0% and 18%) and moderate in F_2^{Lp4} populations (65% and 51%). Thus, the effects of cytoplasm on hybrid breakdown reduce restrictions in re-

Table 2 In vivo pollen germination test, fruit set and seed set data of selected plants from III (F_2^{Le}) and population IV (F_2^{Lp4})

Cytoplasm genotype	% Pollen stainability	*In vivo pollen germination test*			
		Pollen tubes in style[a]	Pollen tubes at the end of style[b]	Fruit set[c]	% Seed germination
Le	84.8	++++	++++	N	–
Le	84.0	+++	+++	Y	25
Le	76.6	++	+++	Y	25
Le	71.3	++++	++++	Y	33
Le	70.9	++++	++++	Y	0
Le	70.4	+++	+++	Y[d]	–
Le	68.2	+++	+++	N	–
Le	66.9	+++	+++	Y	81
Lp	94.7	+++	+++	N	–
Lp	86.6	+++	++++	Y	88
Lp	85.2	++++	+++	Y	86
Lp	83.2	+++	+++	Y	92
Lp	82.6	++++	++++	Y	66
Lp	82.3	++	++	N	–
Lp	81.0	+++	+++	N	–

[a] ++++ All of the flowers tested have pollen tubes growing in the style
+++ More than half of the flowers tested have pollen tubes growing in the style
++ About half of the flowers tested have pollen tubes growing in the style
[b] ++++ In all of the styles, the pollen tubes reach the end of the style
+++ In more than half of the styles, the pollen tubes reach the end of the style
++ In about half of the styles, pollen tubes reach the end of the style
[c] F_2 plant set fruit successfully (Y) or failed to set fruit (N)
[d] F_2 plant set parthenocarpic fruit

production, but do not eliminate them in interspecific F_2s between *L. esculentum*×*L. pennellii*.

Pollen germination

To investigate the nature of non-fecundity in the F_2 populations, in vivo and in vitro pollen germination was tested for selected fecund and non-fecund individuals from population III (F_2^{Le}) and population IV (F_2^{Lp4}) (Table 2). A proportion of the pollen from all of these plants germinated in vitro, indicating that at least some of the pollen from each plant was viable. However, the percentage of in vitro pollen germination for each of the plants and controls varied greatly each time the germination test was performed. A two-way ANOVA test for percent pollen germination applied to the data from the controls (NY, Lp, NY^{Lp4}, F_1^{Le} and F_1^{Lp4}) indicated that interaction between testing date and genotype was highly significant (data not shown). Therefore, in vitro pollen germinability could not be used to quantify reliably the pollen viability of individual F_2^{Le} and F_2^{Lp4} plants.

The same F_2^{Le} and F_2^{Lp4} plants were also self-pollinated to test in vivo self-pollen tube growth. For each plant, at least half of the flowers tested had pollen tubes growing into the style, and most of the styles had numerous pollen tubes at, or growing into, the ovary (Table 2). The method used was not able to determine whether pollen tubes entered the micropyle or whether double fertilization was achieved. However, sufficient pollen tubes were observed reaching the ovary for each of the plants tested to support full fertilization and seed set barring further reproductive barriers.

Discussion

The parental lines NY and NY^{Lp4} were fertile, fecund and did not exhibit any impediment to sexual reproduction. The wild species, Lp, had normal reproductive development but was less productive than NY and NY^{Lp4} in terms of fruit set, seed set and low seed germination rate. Reduction in seed set was due in part to the production of some parthenocarpic fruit. It was also due to differences in floral structure between the two species. The style of NY and NY^{Lp4} flowers is inserted, and pollen dehiscence occurs through the development of stomium (longitudinal slits between the two pollen sacs of each lobe) (Fahn 1982) facing inward towards the stigma (Cooper 1927). In contrast, the style of the Lp flower is exserted, and pollen dehiscence occurs by mean of apical pores at the end of the anthers (Correll 1958). Therefore, based on structural differences, there is less chance for the stigma of an Lp flower to be pollinated by floral vibration.

No reproductive barriers were detected in F_1^{Le} or F_1^{Lp4} plants, although some of the reproductive parameters of F_1^{Le} plants were not as high as those of NY and Lp plants. These results agree with the observations of Rick (1960, 1969). Also, neither the *L. pennellii* cytoplasm nor the segment of chromosome 1 homozygous for *L. pennellii* affected the fertility/fecundity of the F_1^{Lp4}.

Reproductive parameters were reduced in the F_2^{Le} populations. Three to 4% of the plants in the F_2^{Le} populations showed abnormalities in flower or pollen development that were not observed in the parental or F_1 controls. Mean percent pollen stainability was lower for both F_2^{Le} populations than for the parental lines. However, the lack of seed set from self-pollinations of the F_2^{Le} plants was not caused by low percentages of germinable pollen or abnormal pollen germination, since in vivo and in vitro pollen germination tests did not indicate abnormalities or differences in pollen germination and pollen tube growth after self-pollination in the fruitless versus fruitful F_2^{Le} plants. The barriers to flower or pollen development in the F_2^{Le} populations should not result from lack of homology between homologs of the two species, because chromosome pairing in the F_1 hybrid of *L. esculentum*×*L. pennellii* is essentially normal (Khush and Rick 1963). Normal female gamete development in parthenocarpic F_2^{Le} plants was indicated by their ability to produce fruit with viable seed when pollinated with *L. pennellii* pollen. Additionally, self-incompatibility could not account for the lack of self fruit and seed set, since both first generation parents (NY and Lp) and the F_1 were completely self-compatible. Mean percent seed germination of the F_3^{Le} was much lower than that of the NY, F_1^{Le} and F_2^{Le}. Since non-germination is due to non-viability rather than seed dormancy, hybrid breakdown also affected seed development after fertilization. Therefore, the effects of hybrid breakdown on reproduction include plants not able to produce flowers, preanthesis bud abortion, reduced pollen viability and some barrier(s) functioning either immediately before or at fertilization, or very early during embryo development.

F_2^{Lp4} populations also exhibited symptoms of hybrid breakdown, but to a lesser extent than the F_2^{Le} populations. The abnormalities in floral development observed in the F_2^{Le} were largely missing for the F_2^{Lp}. Percentage pollen germination and fruit set was lower in the F_2^{Lp4} populations than in the parental controls, but was higher than in the F_2^{Le} populations. As in the F_2^{Le} populations, no correlation was found between pollen stainability and fruit set in the F_2^{Lp4}. In vivo and in vitro pollen germination tests indicated normal pollen germination and pollen tube growth in the F_2^{Lp4} plants which failed to set self fruit. However, these self fruitless F_2^{Lp4} plants produce fruit and seed when pollinated with Lp pollen. Therefore, the hybrid breakdown observed in the F_2^{Le} and F_2^{Lp4} plants is similar in the developmental stages affected; however, the degree to which it is expressed is significantly different between the two populations.

The different effects of hybrid breakdown in the F_2^{Le} and F_2^{Lp4} populations should be due to genetic differences between these populations. The two parent lines, NY^{Lp4} and NY, are genetically similar, but differ for cytoplasm type and the presence or absence of segregation for loci on chromosome 1 between the markers CT233 and

TG71. Populations I and III (F_2^{Le}) possessed the *L. esculentum* cytoplasm and segregated for markers in the region on chromosome 1, while populations II and IV (F_2^{Lp4}) possessed *L. pennellii* cytoplasm and were homozygous *L. pennellii* for the loci on chromosome 1. If the differences in fertility and fecundity between the F_2^{Le} and F_2^{Lp4} populations are due to the loci on chromosome 1, plants from F_2^{Le} which are homozygous *L. pennellii* for all or the majority of the markers in this region should have the higher fertility and fecundity seen in the F_2^{Lp4} populations. However, examination of F_2^{Le} plants homozygous *L. pennellii* for all, or at least 50%, of this region on chromosome 1 shows that the fertility and fecundity of these F_2^{Le} plants were more characteristic of the F_2^{Le} than F_2^{Lp4} populations. Therefore, there is no indication that the region on chromosome 1 homozygous for *L. pennellii* in the F_2^{Lp4} is related to their increased fertility and fecundity.

An alternative explanation for the difference in the fertility/fecundity of the F_2^{Le} versus the F_2^{Lp4} population is the effect of cytoplasm type. The relative fertility/fecundity levels of NY, NY^{Lp4}, F_1^{Le} and F_1^{Lp4} show that cytoplasm alone does not affect fertility/fecundity. It is most likely that the effect of cytoplasm on the severity of hybrid breakdown involves interaction of the cytoplasm with a nuclear locus (loci) controlling hybrid breakdown. The *L. esculentum* cytoplasm could restrict the transmission of gametes or development of embryos with a homozygous *L. pennellii* genotype at some unidentified nuclear locus (loci). Conversely, the *L. pennellii* cytoplasm is more permissive, allowing the transmission of gametes or development of embryos with similar nuclear genomes. The effect of cytoplasm on hybrid breakdown could be indirect. For example, the cytoplasm could influence segregation of a genomic region that includes a locus controlling hybrid breakdown, and so result in altered levels of fertility and non-fecundity.

Defining the cause of non-seed set in the F_2 populations is complicated because the barrier occurs between fertilization and early embryo development. The paucity of information on events surrounding syngamy and the technical difficulties in observing this complex portion of the reproductive cycle (Linskens 1974) limit what can be deduced. Within the Solanaceae, one barrier that occurs after pollen tubes reach the ovary appears to involve abnormal release of sperm at the embryo sac or problems achieving syngamy (Choudhury 1959a, b; Bannikova and Khvedynich 1974; Gradziel et al. 1993). Postzygotic barriers to embryo and/or endosperm development have also been documented for crosses within *Lycopersicon* species (Smith 1944; Cooper and Brink 1945; Choudhury 1959b; Rick 1963; Hogenboom 1972; Barbano and Topoleski 1984; Smith and Desborough 1986). Identification of the exact stage at which development is restricted will be necessary to better understand hybrid breakdown.

Isocytoplasmic lines may assist breeding programs using wild species as a source of novel traits by increasing the fecundity of F_2 progeny and subsequent transfer of the trait. This is especially important in breeding for multigenic traits, which are unlikely to be transferred by molecular means in the foreseeable future, and which are more difficult to transfer than monogenic traits, particularly if problems with fecundity limit population sizes. Further studies of the developmental stage of hybrid breakdown, the function of genes involved in hybrid breakdown, and the interactions between these genes and cytoplasm will provide a better understanding of the mechanism of hybrid breakdown and help design strategies to facilitate the utilization of wild germplasm in breeding programs.

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