CONTROLLED INTROGRESSION OF CHROMOSOMES OF *SOLANUM PENNELLII* INTO *LYCOPERSICON ESCULENTUM*: SEGREGATION AND RECOMBINATION*

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LYCOPERSICON esculentum Mill. (cultivated tomato) and *Solanum pennellii* Corr. provide ideal material for a study of species hybridization and the effects of introgression. They differ in a great array of characters manifest in every part of their gross morphology and in several aspects of their physiological reaction to different environments. Despite such differences they can be hybridized with little difficulty. Both have 12 pairs of chromosomes, which are nearly identical in morphology, even in the extended condition at pachytene. Furthermore, homologues of F_1 hybrids pair with little or no irregularity, and all stages of meiosis are essentially normal (KHUSH and RICK 1963). Such hybrids are approximately 25% as fertile as the parents in terms of viable progeny yielded after self-fertilization (RICK 1960). Gamete and seed fertility are higher in the 4n than 2n F_1 hybrids. In keeping with such improvement, genetic segregation in their progeny indicates a moderate level of preferential chromosome pairing (RICK and KHUSH 1962). The mean level of fertility declines in F_2 and early backcross generations but never so low as to obstruct seriously further breeding efforts.

This study was facilitated by the recently improved understanding of the genome of the tomato parent. Suitable marker genes can be selected from a total of nearly 1,000 now known; each linkage group has been identified with its respective chromosome and the loci of many key markers have been approximated cytologically by means of induced deficiencies (KHUSH and RICK 1968). This information has assisted in the synthesis of linkage tester stocks with groups of strategically placed genes with well-defined phenotypic expression. Thus provided with chromosome markers, *L. esculentum* is a suitable recurrent parent for backcrosses, which can be made consecutively because the selected mutant genes are recessive. A preliminary note (RICK 1965) was published on this research.

MATERIALS AND METHODS

The tomato parent was represented in these tests by the compound linkage tester stocks listed in Table 1. Information is presented there on the symbol, phenotype, and chromosome for

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TABLE 1

Chromosomal location and phenotypic effects of genes used in linkage tests

Chromosome	Position*	Gene symbol	Gene name	Phenotype
2	52	*Me*	Mouse ears	Leaf excessively subdivided into broad segments
	78	*aw*	without anthocyanin	Anthocyanin completely lacking on all parts
	99	*m*	mottled	Leaf contracted and finely streaked light green
	100	*d*	dwarf	Habit dwarf; leaf stiff, convex, bullate
6	7	*yv*	yellow virescent	Foliage near growing point yellow
	42	*m-2*	mottled-2	leaf finely mottled yellow-green
	66	*c*	potato leaf	leaf with fewer, coalesced segments
8	0	*l*	lutescent	leaves prematurely senescing
	22	*bu*	bushy	internodes very short, rachis elongate
	31	*dl*	dialytic	anthers dialytic; trichomes reduced, distorted
10	70	t^{o}	virescent tangerine	Foliage near growing point yellow-white; fruit flesh bright orange
	90	*ag*	anthocyanine gainer	Anthocyanin absent in seedling, appearing later on dorsal leaf surface
11	17	*j*	jointless	Pedicel articulation lacking
	37	*hl*	hairless	Large trichomes lacking; stems brittle
	57	*a*	anthocyaninless	Anthocyanin completely lacking on all parts

* From revised linkage maps in Report of the Tomato Genetics Cooperative **18:** 5, 6 (1968).

each gene. For each of the chromosomes represented, all of the listed genes have been assembled in a single tester stock. Additional genes for male sterility have been incorporated in some of the stocks to facilitate large-scale hybridization. Figure 1 indicates the relative positions of these markers on linkage maps as ascertained from linkage analysis and cytological study of various

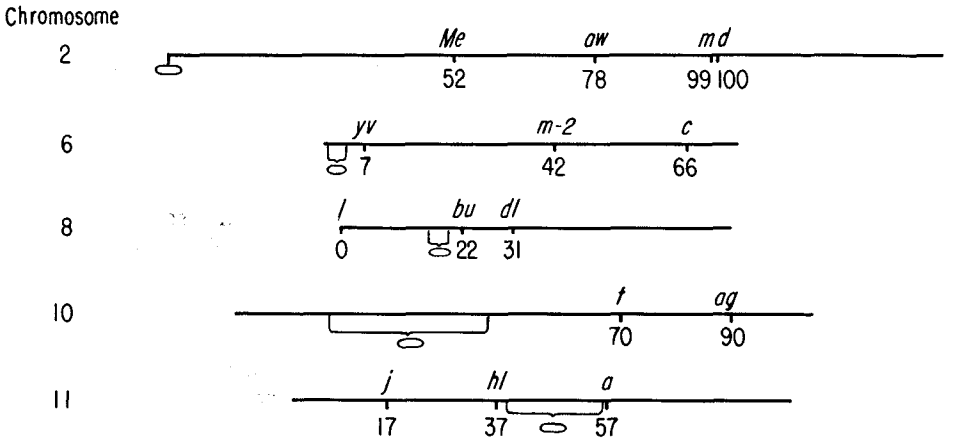

FIGURE 1.—Chromosome maps showing loci of the marker genes used in this investigation (from Report of the Tomato Genetics Cooperative **18:** 5, 6, 1968). The closest approximations to centromere positions are indicated by brackets (KHUSH and RICK 1968).

deviating chromosomal types. Since all of the mutants except *j* manifest their phenotype in the seedling stage, it is possible to score large progenies in a relatively small area.

A single geographic race (LA 716) from Atico, Prov. Arequipa, Peru, was selected for the *S. pennellii* parent. Among all known accessions it is unique for its self-compatibility, which permits selfing and progeny tests of all descendents. Self-fertility and continued existence in a small natural population probably account for the extreme uniformity that has been observed in all populations that we have grown of LA 716.

A severe unilateral incompatibility between the two species requires that *L. esculentum* be used as the pistillate parent in the original hybridization and earlier backcrosses. Reciprocal crosses can be made after continued backcrossing has eliminated the polygenic system that controls the unilateral compatibility (HARDON 1967). Backcrossing to *S. pennellii* is severely impeded by male sterility, the inevitable product of interaction between *esculentum* cytoplasm and various *pennellii* genes (ANDERSEN 1963).

For each of the chromosome series, five different backcross lines were established, each tracing to a different heterozygote in the first backcross generation (BC_1). Comparison of these sublines should permit detection of differential genetic or environmental effects on recombination. In each backcross generation, multiple heterozygotes (plants with normal phenotype) were selected to sire the next generation, and seed remnants were retained for comparative tests of different generations. Backcross lines of pure *esculentum* background served as controls.

Since crossing over is known to be sensitive to environmental factors—for example, mineral deficiencies (GRIFFING and LANGRIDGE 1963)—attempts were made to maintain environmental conditions as uniform as possible. This endeavor was expedited by the use of heterozygotes as staminate parents—an arrangement fortunately permitted by the unilateral barrier. Since sufficient pollen for extensive crosses can be produced by a few plants or even a single plant, it is feasible to confine all of the heterozygotes to be used for a given series in a relatively small space. They were grown under optimal light and nutritional conditions in the central area of a greenhouse in which temperatures were maintained, by a combination of heating and cooling devices, within a 17–21°C range at night and 24–30°C range in daytime. These plants were frequently rotated in position in efforts to equalize the environment. Crosses with all parents of a series were made within a 1–2 hr period, and all crosses were date-marked. The pistillate parents were usually grown under the same conditions, although for certain series they were planted in the open field. The plants of a single parent were always maintained under the same conditions.

An additional recombination test was made by planting excised embryos on sterile culture medium. For this purpose parental lines were grown and hybridized in the same fashion as in the tests outlined above. Five weeks after pollination, part of the fruits from each pistillate parent were harvested, sterilized in sodium hypochlorite, and the embryos excised from developing ovules, any attached endosperm being allowed to remain with the embryo. The cultures were kept in deep Petri dishes, in which the supporting medium was either glass wool or standard germination blotting paper. The culture fluid consisted of Hoagland's solution (essential minerals) and 0.5% sucrose. All components were autoclaved. The cultures were maintained in an incubator held alternately at 25°C for 12 hr illumination under banks of fluorescent lamps and at 20° for a 12 hr dark period. Under these conditions the embryos sprouted and developed rapidly into seedlings. As soon as radicles had emerged sufficiently, the seedlings were transplanted into sterilized soil in the greenhouse and tended thereafter in the same fashion as the soil cultures. Other fruits from the same batches were allowed to ripen normally (8–9 weeks), seeds were extracted, and progenies were planted according to our standard methods.

RESULTS

Monogenic segregation: Segregation of the fifteen selected genes in the alien substitution backcrosses was characterized by substantial deviations from proportions observed in the *esculentum* controls as well as from the expected 1:1

TABLE 2

Summary of monogenic segregations in BC_1, BC_3, and BC_5

Chromosome	Gene	BC_1			BC_3			BC_5			*esculentum* control		
		Total	Number mutant	Percent mutant	Total	Number mutant	Percent mutant	Total	Number mutant	Percent mutant	Total	Number mutant	Percent mutant
2	*Me*	1,099	568	51.7	...	...	...	...	...	...	1,367	718	52.5
	aw		579	52.7	1,283	640	49.9	1,801	913	50.7	582	279	47.9
	m		...	...		648	50.5		900	50.0		273	46.9
	d		574	52.3		650	50.7		900	50.0		274	47.1
6	*yv*	358	173	48.3	284	114†††	* 40.1	338	167	49.4	179	92	51.4
	m-2		165	46.1		121†	42.6		183	54.1		100	55.9
	c		159†	44.4		134	47.2		190†	56.2		95	53.1
8	*l*	542	327†††	60.3	1,215	856***	70.5	545	399***	73.2	983	544†††	55.3
	bu		335***	61.8		866***	71.3		402***	73.8		546†††	55.5
	dl		333***	61.4		855***	70.4		394***	72.3		548†††	55.7
10	t^v	294	89***	30.3	310	151	48.7	605	264†††	43.6	540	242†	44.8
	ag		104†††	** 35.4		164*	52.9		274*	45.3		250	46.3
11	*j*	1,029	377***	36.6	1,246	498***	40.0	1,002	314***	31.3	1,352	706	52.2
	hl		375***	36.4		503***	40.4		321***	32.0		677	50.1
	a		375***	36.4		493***	39.6		333***	33.2		700	51.8

* Deviation from control significant at 5% level.
** Deviation from control significant at 1% level.
*** Deviation from control significant at 0.1% level.
† Deviation from 1:1 significant at 5% level (specified only if different from control test).
†† Deviation from 1:1 significant at 1% level (specified only if different from control test).
††† Deviation from 1:1 significant at 0.1% level (specified only if different from control test).

ratio (Table 2). A prevailing lack of heterogeneity between subfamilies permitted pooling the data for each series. The most extreme and consistent deviations were observed for the chromosome 8 and 11 series. In the former the ratios approximated 1 normal : 2 mutant; in the latter, 2 normal : 1 mutant. The deviations for markers on the same chromosome are remarkably alike—a result expected because, as outlined in the following section, these markers recombined to a very limited extent. Control segregations for chromosome 8 were distorted in the same direction but to a much lesser extent. Mutant segregants were deficient in BC_1 and BC_5 of t^v and *ag*, but the opposite trend was encountered for *ag* in BC_3, the difference being significant only at the 5% level. For chromosome 2 no significant deviations were found, and the few for chromosome 6 so closely bordered on significance that the deviations cannot be considered well established.

Since the heterozygous parents for each of these tests were staminate, differential fertilization by male gametes, which in higher plants are notoriously prone to selective elimination, might account for part or all of the observed deviations. Tests were accordingly made between reciprocal crosses of controls and BC_6 introgressants. The chromosome 8 and 11 series were selected because their monogenic ratios had been found to be distorted to the greatest extent (Table 2). Two independent subfamilies were maintained for each series. All tests were made at the same time and all heterozygous parents were grown in the above-specified uniform environment. Except for a failure of reciprocal control crosses in the chromosome 8 series, adequate data were obtained for all series.

The results of the reciprocal tests are summarized in Table 3. The proportion of recessive segregants in the direct (male-heterozygous) BC_6 tests deviates in the same direction and to the same extent for all tested mutants of both chromosomes as it did in the previous experiment (Table 2). All deviations from 1:1 are highly significant.

For chromosome 8 the direct controls deviate in the same direction but to a lesser degree, the differences from 1:1 only bordering on statistical significance. The agreement between direct BC_6 subfamilies permits pooling of the data for each gene. Excess mutants were also found in BC_6 reciprocals, but the differences were much smaller than those of the direct crosses. The departure from 1:1 is significant for all genes of subfamily 1, but for none in subfamily 2; moreover, tests of heterogeneity between subfamilies were significant at the 1 and 5% levels, precluding the pooling of these data. Of special importance is the very highly significant difference between direct and reciprocal crosses in every paired comparison.

Results with markers of chromosome 11 differ strikingly. In contrast, the reciprocal crosses deviate nearly to the same extent as the direct BC_6. The lack of intersubfamily heterogeneity permits pooling of the data for direct as well as reciprocal backcrosses. In all comparisons between direct and reciprocals, within family or pooled data, the χ^2 tests show no heterogeneity except for the pooled data of *hl* and that only at the 5% level. Segregation for *a* and *hl* therefore tends to be somewhat less distorted when the female parent is heterozygous, but the

TABLE 3

Comparison of monogenic ratios in direct and reciprocals of the sixth backcross

Category	Chromosome 8						Chromosome 11				
		l		*bu*		*dl*		*a*		*hl*	
	Total	Number	Percent	Number	Percent	Number	Percent	Total	Number	Percent	Number	Percent
Dir. BC_6, subfam. 1	1,018	713	70.0	714	70.1	712	69.9	412	155	37.6	150	36.4
χ^2, dir. BC_6, subfam. 1 vs. 1:1		163.5***		165.9***		161.9***			24.7***		36.9***	
Dir. BC_6, subfam. 2	1,227	883	72.0	881	71.8	882	71.9	256	94	36.7	90	35.1
χ^2, dir. BC_6, subfam. 2 vs. 1:1		235.9***		232.4***		234.1***			17.5***		22.0***	
χ^2, dir. BC_6, subfam. 1 vs. 2		0.9		0.7		0.9			0.2		0.05	
Rec. BC_6, subfam. 1	581	337	58.0	327	56.3	335	57.7	446	185	41.5	189	42.4
χ^2, rec. BC_6, subfam. 1 vs. 1:1		14.6***		8.9**		13.3***			12.6***		10.1**	
Rec. BC_6, subfam. 2	1,085	554	52.1	553	52.0	562	52.8	254	107	42.1	107	42.1
χ^2, rec. BC_6, subfam. 2 vs. 1:1		0.5		0.4		1.3			6.0*		6.0*	
χ^2, rec. BC_6, subfam. 1 vs. 2		7.0**		4.1*		5.0*			0.008		0.0002	
χ^2, dir. vs. rec. BC_6, subfam. 1		23.2***		30.6***		24.1***			1.2		2.9	
χ^2, dir. vs. rec. BC_6, subfam. 2		106.1***		105.2***		98.2***			1.3		2.3	
Pooled dir. BC_6	2,245	1,596	71.1	1,595	71.0	1,594	71.0	668	249	37.2	240	35.9
Pooled rec. BC_6		...	...		...		...	700	292	41.7	296	42.3
χ^2, dir. vs. rec. pooled BC_6		....		....		....			2.6		5.5*	
Dir. control	453	247	54.5	249	55.0	253	55.8	403	218	54.1	212	52.6
χ^2, dir. control vs. 1:1		3.5		4.3*		6.0*			2.5		...	
χ^2, pooled dir. BC_6 vs. dir. control		47.0***		44.3***		39.4***			28.2***		28.0***	
Rec. control		...	...		...		...	310	148	47.7	146	47.1
χ^2, rec. control vs. 1:1		....		....		....			0.5		0.9	
χ^2, pooled rec. BC_6 *vs.* rec. control		....		....		....			2.9		1.8	

Dir. = direct (male = heterozygous); Rec. = reciprocal.
* Deviation significant at 5% level.
** Deviation significant at 1% level.
*** Deviation significant at 0.1% level.

TABLE 4

Summary of recombination values for chromosomes 8, 10, and 11

Chromosome	Interval	Pedigree	Number of families†	Total progeny	Recombinants		χ^2	
					Number	Percent	*vs.* control	Heterogeneity
8	*l–bu*	BC_1	2	542	26	4.8	24.16***	0.52
		BC_3	4	1215	30	2.5	86.69***	0.53
		BC_5	3	545	17	3.1	37.74***	3.06
		BC_{1-5}	9	2302	73	3.2	75.36***	5.92
		con.	3	983	126	12.8	....	2.81
	bu–dl	BC_1	2	542	16	2.9	21.83***	0.22
		BC_3	4	1215	16	1.3	75.98***	0.29
		BC_5	3	545	8	1.5	35.58***	0.40
		BC_{1-5}	9	2302	40	1.7	71.8 ***	2.12
		con.	3	983	94	9.6	....	2.55
10	*ag–t*^{v}	BC_1	2	838	172	20.1	0.83	3.00
		BC_3	2	1139	143	12.6	16.55***	0.13
		BC_5	3	920	71	7.8	51.97***	0.54
		BC_{1-5}	7	2897	386	13.3	....	51.51***
		con.	2	1187	223	18.8	....	0.55
11	*j–hl*	BC_1	3	1029	126	12.2	0.43	0.72
		BC_3	4	1246	103	8.3	16.06***	10.11*
		BC_5	4	1002	87	8.7	11.47***	2.34
		BC_{1-5}	11	3277	316	9.6	....	22.29*
		con.	2	1352	179	13.2	....	0.05
	hl–a	BC_1	3	1029	110	10.7	30.37***	1.29
		BC_3	4	1246	78	6.3	92.69***	1.60
		BC_5	4	1002	74	7.4	63.38***	6.19
		BC_{1-5}	11	3277	262	8.0	....	22.49*
		con.	2	1352	257	19.0	....	0.71

† Each family descended from a different BC_1 segregant.
* Difference significant at 5% level.
** Difference significant at 1% level.
*** Difference significant at 0.1% level.

differences are of a much smaller magnitude than those obtained for markers of chromosome 8.

Recombination: Linked intervals on chromosomes 8, 10, and 11 were subjected to the first and most intensive tests because they were the best marked of any regions at the time the investigations were started. Simultaneous tests were made of BC_1, BC_3, and BC_5 *esculentum* controls on several sublines for each series. In spite of all efforts, we were unable to keep five sublines throughout each series, but a minimum of two were always maintained. The results are summarized in Table 4. Test and control frequencies are compared by means of 2×2 contingency χ^2 tests, and, for each interval, consistency within and between backcrosses is tested by heterogeneity χ^2.

The reliability of these recombination tests is manifest in the low level of heterogeneity indices. Of the 20 groups of data (five control groups and three backcrosses each of the five tested intervals) heterogeneity is revealed for only

one (BC_3 for *j-hl*) and that only at the 5% level of significance. Thus, under the regime of these tests, neither environmental nor genetic differences within specific groups were sufficient to be detected. Pooling of the data within each of the 20 groups is thereby warranted.

A different situation is presented by tests *between* different backcrosses: significant differences exist among all BC's for the *ag–t*^{v} interval and between BC_1 and BC_3 and between BC_1 and BC_5 but not between BC_3 and BC_5 for both tested intervals of chromosome 11. Since no appreciable environmental heterogeneity had been detected, it is logical to conclude that genetic changes account for the inconsistencies between backcrosses, and pooling cannot be justified. The reality of such differences is underscored by the uniform tendency in each interval for recombination to diminish from the highest values in BC_1 to lower levels in BC_3 and BC_5, between which differences are not significant. The extent of this decrease varies from 70% of the BC_1 level for *j–hl* to 48% for *bu–dl*. Apparently reconstitution of the *esculentum* genotype, already fairly well achieved by BC_3, accounts for such trends. The lack of appreciable change between BC_3 and BC_5 suggests that tests need not be continued beyond BC_3 in order to adequately determine recombination levels and trends.

Whereas differences between sublines are insignificant and those between backcrosses are of moderate degree, those between the interspecific backcrosses and controls are of a much larger order: recombination values for 14 of the 15 test groups are smaller than those of the respective controls, and, for 13 comparisons, the differences are significant at the 0.1% level. The greatest modification is observed for the chromosome 8 intervals, in which the values for BC_3 and BC_5 amount to only 10–20% of those of the controls.

Comparison of chi-squares reveals the magnitude of the differences in recombination between hybrid and control families. Thus, for the chromosome 8 intervals, the chi-squares for comparison with controls range from 7 to 27 times those for heterogeneity—in effect, the error component—of both groups. Comparable differences exist in the *hl–a* tests and for BC_5 of the other two tested intervals.

The frequency of double crossovers was recorded throughout these tests, but since it is negligible in these instances of reduced recombination, the data are not presented. In the controls and the data of other tests the frequency of double recombination falls within the expected range and the recombination represented in the double is reported for each interval in the tables.

Backcrosses for the chromosome 8 and 11 series were continued to BC_6 for the purpose of comparing recombination in reciprocal crosses. Genes controlling the unilateral incompatibility barrier were eliminated during the course of these backcrosses because complete compatibility was experienced in the reciprocals. This test was conducted in the same fashion as the preceding series in respect to maintainance of sublines and control of environment. It is the same test as that for which monogenic segregation was presented in Table 3.

Internal consistency of the results is again revealed by the lack of intersubfamily heterogeneity. Further, the reductions in recombination of the BC_6 direct crosses are of the same degree as those found previously for the three tested

TABLE 7

Recombination rates for chromosome 2

Generation	Total	*Me–av* Recombinants Number	Percent	χ^2 *vs.* control	Total	Interval *av–d* Recombinants Number	Percent	χ^2 *vs.* control	Total	*d–m* Recombinants Number	Percent	χ^2 *vs.* control
BC_1	1099	200	18.2	1.2	1099	101	9.2	14.5***	*m* not segregating			
BC_3		†			1283	88	6.9	5.4*	1283	6	0.47	n.s.
BC_5		†			1801	100	5.5	2.0	1801	3	0.17	n.s.
BC_3+BC_5		†			3084	188	6.1	3.7	3084	9	0.29	n.s.
Control (*esc.*)	1367	273	20.0		582	23	3.9	..	582	1	0.17	

* Deviation significant at 5% level.
*** Deviation significant at 0.1% level.
† Since *Me* is a dominant marker it can be tested only in BC_1.

TABLE 8

Recombination rates for chromosome 6

Generation	Interval							
		yv–m-2 Recombinants				*m-2–c* Recombinants		
	Total	Number	Percent	χ^2 *vs.* control	Total	Number	Percent	χ^2 *vs.* control
BC_1	358	8	2.2	70.1***	358	70	19.5	6.0*
BC_3	284	17	6.0	34.5***	284	53	18.7	2.3
BC_5	338	34	10.1	20.7***	338	53	15.7	6.2*
Control (*esc.*)	179	46	25.7	. . .	179	45	25.1	. .

* Deviation significant at 5% level.
*** Deviation significant at 0.1% level.

lines are significant. The extent of decrease is much greater for the proximal interval, *yv–m-2*, than for the distal one, *m-2–c*. No consistent trend is clear in the successive backcrosses.

The fit of monogenic ratios to 1:1 is good for chromosome 2 markers, while, for those of chromosome 6, deviations in favor of wild alleles were found in BC_1 and BC_3 but in favor of *esculentum* alleles in BC_5. In no case, however, are the distortions so large as those observed for markers of chromosomes 8 and 11 (Table 2).

The data for chromosomes 2 and 6, although more fragmentary and less conclusive than those for 8, 10, and 11, do reveal that hybrid recombination rates can approximate normal rates for at least one interval. Altogether, these experiments have demonstrated that recombination rates in the *esculentum-pennellii* hybrid and its derivatives can vary, according to the chromosome segment, from 10 to 100% or more of the control values.

DISCUSSION

According to the results obtained here, it is feasible to arrange experiments with tomato plants so that moderate differences in recombination can be detected. In this fashion it has been demonstrated that the substitution of certain *pennellii* chromosomes or portions thereof can lead to drastic reductions below *esculentum* levels while for others the consequences are moderate or too small to be detected.

The major problem posed by these findings is to account for such decreased recombination. Attention should first be called to the nearly normal cytological behavior of the F_1 hybrid (KHUSH and RICK 1963). Twelve bivalents are regularly formed, and even in respect to chiasmata counts, the F_1 falls only slightly below its parents. Essentially the same situation holds for all backcross derivatives whose chromosome cytology has been examined—a finding that is compatible with the high level of their gamete and seed fertility. Assuming the commonly accepted tenet of cytogenetics that crossing over leads to the formation of chiasmata, we would anticipate normal or nearly normal rates of crossing over in the F_1 and derivatives. Clearly, then, whatever process or processes are responsible

for the decreased recombination must be superimposed upon a crossing over situation that is essentially normal, at least in total amount.

Likewise, arguments that reduced crossing over might account for the reduced recombination in only a few bivalents of the set cannot be supported. Even though the hybrid has a total chiasmata count slightly lower than that of its parents, every bivalent has at least one chiasma and the majority have two. More tenable is the hypothesis that total crossing over per bivalent is normal but the frequency has been repatterned to be higher in some parts but lower in others. In introgressants in which chromosome segments of *Gossypium armourianum* and *G. raimondii* were substituted in a background of *G. hirsutum*, RHYNE (1960) found reduced recombination within substituted segments associated with increased recombination in the adjacent, pure *hirsutum* regions. In the present example it might be supposed that decreased crossing over in the proximal regions of chromosomes 6, 8, and 11 be balanced by higher rates in more distal regions. The data show that for the long arm of chromosome 6 the hybrid rates for the proximal interval, *yv–m-2*, are markedly more diminished than those for the distal *m-2–c* section; for chromosome 8 the decrease is extreme and nearly the same for both the centromere-spanning *l–bu* interval and the nearby, proximal *bu–dl*; for chromosome 11 the degree of reduction is somewhat larger for the *hl–a* region, which includes the centromere, than for *j–hl*, which occupies the proximal half of the mapped portion of the short arm (Figure 1). The tests of chromosomes 2 and 10 are not critical because for the former they are confined to the middle part of the long arm, and for the latter only a single interval was tested. Thus, for the critical tests, the results are compatible with this hypothesis to the extent that recombination is reduced more in the proximal or more proximally located sections than in distal ones. Two possibilities thus exist: either (1) such compensation does not occur in our material or (2) it does occur but the compensating regions are distal to any that we have tested. It is hoped that the current amplification of the tomato linkage maps will provide an opportunity to explore the more distal regions in the near future.

Differences between the pachytene chromosomes of the parental species are observed only in the proximal heterochromatin and a few knobs in the euchromatin (KHUSH and RICK 1963). Such heteromorphisms are thought to cause the occasional disturbances of pairing observed in the centric regions—the only meiotic abnormalities of the F_1 hybrid. The cytological findings are therefore in accord with those of genetic recombination.

According to an alternative hypothesis, the basic rate of crossing over in the F_1 and derivatives is not basically different from that of *L. esculentum*, but differential elimination of recombinants might account for the observed reductions. Support for this hypothesis is lent by data on reciprocal crosses for intervals of chromosomes 8 and 11. When heterozygotes were used as female parents, recombination values were considerably higher than in reciprocals, but in all cases remained below control values, significantly so for chromosome 8 intervals. Thus, differential survival of male gametes can account for part but not all of the decrease. Levels of sterility are sufficiently high in BC_1 to permit some post-

syngamic elimination, but tests of critical intervals on chromosome 8 in BC_7 did not show any differential elimination in late embryogenesis and seed germination, and the seed fertility of later backcrosses is generally too high to allow any substantial postsyngamic elimination. Differential survival of recombinants either in gametes or zygotes can therefore explain only part of the total reduction in recombination rates. The virtue of distinguishing between crossing over and recombination in these tests is thus underscored.

That elimination of male gametes accounts for part of the recombinational disturbance in this tomato hybrid is at variance with the conclusion of SMITH (1952) that it is relatively unimportant in restricting gene recombination in F_2 *Nicotiana langsdorfii* × *N. sanderae*. This apparent contradiction might be reconciled in several ways. The tomato data deal with selection in *any* stage of male gametophyte development, whereas the Nicotiana case dealt solely with pollen abortion. Further, the former treats only *intrachromosomal* recombination, whereas the latter, *total* "recombination." Doubtless recombination between chromosomes constitutes a large—probably major—share of the total in interspecific hybrids. The literature on species hybrids is fraught with confusion engendered by failure to distinguish between the two kinds of recombination.

According to the tests of reciprocal crosses, part of the disturbance of recombination can be attributed to some kind of selective elimination of male gametes. The high proportion of stainable pollen in heterozygotes of later backcross generations discounts pollen abortion as a factor, but does not militate against dysfunction of certain classes of stainable pollen grains. Such differential activity could occur at pollen germination or tube growth—stages for which so many examples of selection are known in higher plants. At whatever stage selection acts, the differential might result from greater potency of the genetically more balanced non-recombinant male gametophytes or possibly from a detrimental effect they might exert on recombinants akin to the pollen killer phenomenon discovered by CAMERON and MOAV (1957) in Nicotiana introgressants.

A version of the meiotic drive phenomenon offers a third hypothesis. According to this proposal, poleward movement of non-recombinant chromosomes would be favored over that of recombinants, necessarily at the second meiotic division. In the F_1 and early backcrosses, the non-recombinants from many different dyads would tend to migrate to the same pole. Gametes receiving recombinant chromosomes would be subject to abortion or various degrees of dysfunction. Whilst the process might direct recombinant complements to the degenerating megaspores in female gametogenesis, no such elimination regularly occurs on the male side. The meiotic drive hypothesis thus requires a multiplicity of assumptions, none of which has yet been proved. Until more supporting evidence can be provided, it must be considered less tenable than the other hypotheses.

The relationship between monogenic segregation and recombination deserves attention. Significant disturbances to both have been encountered in these tests, and, if only extremes are compared, the strongest modification of one is accompanied by the greatest deviation of the other (chromosome 8) whilst the closest approach to normality for one is associated with the same for the other (chromo-

some 2). But this semblance of correlation disappears when all series are plotted, even if deviations from 1:1 are treated as absolute values.

The same conclusion is supported by a close comparison of monogenic *vs.* recombination data for chromosome 8, in which the distortions were most extreme. Here, on one hand, the selection in favor of *esculentum* alleles would not have been nearly intense enough to account for the reduced recombination; while on the other, there is no *a priori* reason why an extreme selection against recombinants should favor *esculentum* alleles.

It is noteworthy in this connection that the departures from 1:1 (or controls) in the monogenic ratios are both positive and negative, the most frequent being a deficiency of the *esculentum* (recurrent parent) alleles. Such a trend is contrary to expectation and to the majority of observations in species hybrids, as exemplified by STEPHENS' (1949) report of consistent excesses of the recurrent parent alleles in backcrosses of F_1 *Gossypium barbadense* × *G. hirsutum*. No reason is apparent for the reverse tendencies in the tomato hybrid. Since the selection of male gametophytes is more intense in the modifications of monogenic segregations than in modifications of recombination, the answer probably lies in the strange exigencies of the interaction between stylar tissue and the assortment of male gametophytes with which it is challenged.

Whatever the fundamental nature of these phenomena, the *esculentum-pennellii* hybrid provides another good example of linkage between morphology and viability in plant species. GRANT (1967) reviewed known instances of this phenomenon and discusses its impact on introgression. In the present instance such linkages would tend not only to favor certain alleles, but also to preserve, in some instances in a very highly efficient manner, large linkage blocks of the introgressant species. The tendency to preserve and favor such groups of genes would greatly reduce the assortment of germplasm of the two species and by the same token greatly impede the efforts of the breeder, who generally prefers to transfer small linkage blocks, often single genes.

SUMMARY

Hybrids between *Lycopersicon esculentum* and *Solanum pennellii* were successively backcrossed to the former in order to investigate segregation and recombination. Separate backcross series were made to *esculentum* stocks in each of which a single pair of chromosomes was marked with linked recessive genes. Such series were conducted to BC_5 for 15 genes bounding ten intervals on five chromosomes. Parallel series were maintained in pure *esculentum* background as controls. Strict unilateral incompatibility required use of heterozygotes as male parents in early backcross generations.—Nearly all the monogenic segregations for chromosomes 8, 10, and 11 deviated from 1:1 and control ratios, while those of chromosomes 2 and 6 did not. The deviations, tending to be consistent throughout all backcrosses of a series, varied from 30 to 74% recessive homozygotes. When heterozygotes were used as female parents in reciprocal crosses of BC_6, ratios were similarly distorted, but to a lesser extent.—Precision of the recom-

bination tests was revealed by the lack of heterogeneity in tests among sublines in each series. Recombination diminishes significantly in most series from BC_1 to BC_3, but tends to remain near the same low level to BC_5. The interspecific backcrosses approximated control values for chromosome 2, but for intervals on other chromosomes deviated to as low as 14% of control values, the differences being highly significant. Recombination was lower in centric or proximal than distal regions—a finding that is compatible with the occasional disturbances observed cytologically in chromosome pairing in the centric regions but not elsewhere. According to reciprocal crosses in BC_6, recombination rates were significantly lower when heterozygotes were used as male parents. Observations of developing ovules and comparisons of rates in soil *vs.* sterile cultures revealed little, if any, elimination during maturation of female gametophytes, embryogenesis, or seed germination. Selective elimination of male gametes is thereby implicated for part, but not all, of the reduction in recombination. The data do not reveal any close or causal relationship between distorted monogenic ratios and lowered recombination rates.

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