SEED COLLAPSE FOLLOWING MATINGS BETWEEN DIPLOID AND TETRAPLOID RACES OF LYCOPERSICON PIMPINELLIFOLIUM[1]*

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INTRODUCTION

THE seeds arising from reciprocal matings between tetraploid *Lycopersicon pimpinellifolium* (2n=48) and the common diploid type (2n=24) are abortive and non-functional. This behavior appears to be characteristic of crosses between diploids and their respective autotetraploids. BLAKESLEE, BELLING, and FARNHAM (1923), for example, found that most of the seeds resulting from the 4n×2n cross in *Datura stramonium* were non-viable, and JORGENSEN (1928) observed the same result in matings between tetraploid and diploid races of *Lycopersicon esculentum*. Following the 2n×4n cross in *Zea mays*, according to RANDOLPH (1935), about 98 percent of the seeds are abortive; and of those which are relatively well filled, only a few are germinable. The 4n×2n cross in maize gives an even greater proportion of poorly developed seeds, although the germinating capacity of these seeds was found to be somewhat higher than in the case of the reciprocal.

While seed abortion is not the only manifestation of incompatibility between diploids and their tetraploid derivatives, it is probably the most effective barrier to hybridization between these races. BUCHHOLZ and BLAKESLEE (1929) have shown that abnormal pollen tube development prevents fertilization in 2n×4n matings of *Datura stramonium*. RANDOLPH (1935) found that pollen tube growth is retarded in the corresponding cross in *Zea mays*. The restriction associated with pollen tube growth, however, does not prevent interbreeding between diploids and their respective autotetraploids because it does not apply in the reciprocal cross, 4n×2n. On the contrary, RANDOLPH (1935) observed in matings of Zea which permitted recognition of the two expected classes of seeds that pollen from the diploid is much more effective in accomplishing fertilization than that from the tetraploid when mixtures of the two pollens are applied to the silks of tetraploid plants.

The incompatibility between 4n races and their diploid progenitors is particularly interesting in that it may arise wholly as a result of the difference in chromosome number. The usual failure of the seeds to attain a germinable

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condition following both 2n×4n and 4n×2n crosses is the more remarkable in view of the normal capacity for vegetative growth of the autotriploid plant which may occasionally result from these matings. Obviously the failure is not a function of triploidy as such but arises from the conditions surrounding the triploid embryo within the seed. The nature of the block to the continued growth of diploid-tetraploid hybrids which is interposed at the seed stage is the subject of the present investigation.

MATERIALS AND METHODS

The diploid and tetraploid races of *L. pimpinellifolium* used in this study were described by LINDSTROM (1932), who generously provided us with foundation seed. Both stocks originated from an inbred strain of the Red Currant variety and hence may be expected to carry approximately the same genes. We are indebted to DR. B. L. WADE, U. S. VEGETABLE BREEDING LABORATORY, Charleston, S. C., for seed of the strain of *Lycopersicon peruvianum* (2n = 24) which was employed.

Reciprocal matings between the 2n and 4n races of *L. pimpinellifolium* were made in the greenhouse, the usual precautions being taken to control pollination. Both these stocks also were crossed with *L. peruvianum*, using the latter as pollen parent. The results reported by LESLEY and LESLEY (1943) and by SMITH (1944) indicate that the production of functional embryos following the mating *L. esculentum*×*L. peruvianum* varies somewhat depending upon the *peruvianum* (and, perhaps, *esculentum*) strain used. The *peruvianum* stock here employed had been found at the Charleston laboratory to give only abortive seed in crosses with the common tomato. Since *L. esculentum* is fertile with *L. pimpinellifolium*, it was expected that the 2n *L. pimpinellifolium*×*L. peruvianum* cross likewise would give non-functional seeds. This proved to be the case. The controls used were 2n×2n and 4n×4n crosses in *L. pimpinellifolium*.

The matings made comprise two series, as given below, within each of which it was desired to make comparisons of seed development. The chromosome numbers in cells of the maternal tissue, endosperm and embryo of the seeds resulting from the six matings are shown.

	CHROMOSOME NUMBERS IN SEED		
	MATERNAL TISSUE	ENDOSPERM	EMBRYO
(a) 2n×2n, *L. pimpinellifolium*	24	36	24
2n×4n, *L. pimpinellifolium*	24	48	36
2n *L. pimpinellifolium*×2n *L. peruvianum*	24	36	24
(b) 4n×4n, *L. pimpinellifolium*	48	72	48
4n×2n, *L. pimpinellifolium*	48	60	36
4n×2n *L. peruvianum*	48	60	36

The inclusion in each series of a mating involving *L. peruvianum* as a pollen parent makes possible a direct comparison of seed failure associated with the

2n−4n chromosome difference with that resulting from outcrossing to a distinct species. It would have been desirable to include in series (b) a mating with tetraploid *L. peruvianum*, as male parent. A 4n stock of *L. peruvianum*, however, was not available.

Fruits arising from the matings listed were collected at the following times after pollination: 24, 48, 96, 144, 192, 288 and 384 hours. The fruits were killed in Karpechenko's solution and partially dehydrated by moving them through a series of alcohols up to 70 percent. The seeds were then dissected out and prepared for histological study using the methods earlier described for similar work with other species (COOPER and BRINK 1940). The fruits from a parallel set of matings were allowed to remain on the plants to maturity, at which time they were taken for observations on the frequency of seed and fruit formation.

TERMINOLOGY

For convenience the various classes of seeds under study are designated in accordance with the type of mating from which they have arisen. Thus "2n×2n seeds" are the product of a cross between two diploid *L. pimpinellifolium* plants. Those resulting from mating tetraploid *L. pimpinellifolium* with diploid *L. pimpinellifolium*, the latter being used as the staminate parent, are referred to as "4n×2n seeds," and so on. The seed arising from outcrossing the tetraploid and diploid strains of *L. pimpinellifolium* to *L. peruvianum* are designated "4n×*L. peruvianum*" and "2n×*L. peruvianum*," respectively. Throughout the paper the stock used as the pistillate parent in a cross is written first.

FRUIT AND SEED FORMATION

The data on fruit development are summarized in table 1. All but four of the 63 flowers pollinated in the 2n×2n matings formed mature fruits. The results of these control crosses show that the conditions under which the plants were being grown were favorable for fruit development and that the amount of injury to the flowers in castration was not large. The frequency of fruit development was 90 percent in the 2n×*L. peruvianum* cross. The 2n×4n matings, on the other hand, were barren, the flowers and young fruits all being shed within eight days after pollination. Only about one-half the pistillate flowers used in the 4n×4n matings were represented by fruits at maturity. The initial set was high in this case, but immature fruits were shed at intervals up to 24 days. About 76 percent of the 4n×2n crosses gave rise to mature fruits. The rate of fruiting was high also in the 4n×*L. peruvianum* mating, although counts were not made.

The data on seed formation following the several types of matings are brought together in table 2. The "shrivelled" class comprises the seed-like bodies within the mature fruit which are obviously non-functional. This class is not entirely definitive because of gradations in size of the seed-like structures, and the extent to which it represents the seeds which begin to grow but

whose development is prematurely arrested can only be surmised. It is very probable, however, that the remnants of seeds collapsing during the first few days after fertilization are not large enough to be included in it.

The 2n×2n matings gave 26.8 plump and less than one shrivelled seed per ovary, on the average. Since the number of ovules per ovary averaged 29.4, based on counts of 33 ovaries, this approximates a full set of seed. The 2n×*L.*

TABLE 1

Mature fruit development in matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum.

MATING	NUMBER		PERCENTAGE MATURE FRUIT
	FLOWERS POLLINATED	FRUIT MATURED	
2n×2n	63	59	93.6
2n×*L. peruvianum*	62	57	90.3
4n×4n	18	10	55.6
4n×2n	33	25	75.7

TABLE 2

Seed production after matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum.

MATING	NO. OF FRUITS	NO. OF SEEDS		AVERAGE PER FRUIT	
		SHRIVELLED	PLUMP	ALL SEEDS	PLUMP SEEDS
2n×2n	59	35	1583	27.4	26.8
2n×*L. peruvianum*	55	777	2*	14.2	.04
4n×4n	12	50	95	12.1	7.9
4n×2n	9	225	1**	25.1	.1
4n×*L. peruvianum*	9	97	140	26.3	15.6

* Both occurred in one fruit; seed large, probably accidental selfs.
** Small.

peruvianum mating, on the other hand, yielded only two plump seeds in 55 fruits. Because these two seeds were large and occurred in a single fruit, it is likely that they resulted from accidental self-pollination. The remaining seeds from this mating were shrivelled and averaged 14.2 per fruit. All the fruits from the 2n×4n mating fell before maturity, as noted above.

The number of plump seeds per ripe fruit averaged only 7.9 for the 4n×4n mating. There were in addition about four shrivelled seeds per fruit. All seeds averaged 12.1, as against 35.2 ovules, per ovary. All of the 226 seeds but one in the nine fruits resulting from the 4n×2n mating were shrunken. The constitution of the single small but plump seed obtained was not determined. The 4n×*L. peruvianum* mating is the most fertile in series (b). Nearly one-half

the ovules gave rise to well developed seeds. The germinability of these seeds has not been adequately tested. One hybrid 4n×*L. peruvianum* plant which was reared, however, made an exceedingly luxuriant growth.

The differences in seed production following the above matings are due in part to non-fertilization of ovules and in part to the failure of fertile ovules to develop into plump seeds. The data in table 3 provide a basis for estimating the relative importance of these two factors. Numerous observations on sectioned tomato material show that the ovule enlarges but little beyond the size attained at the mature embryo sac stage unless it becomes fertile. Hence a

TABLE 3

Average number of ovules per ovary enlarged at 12 days after pollination and plump seeds at maturity of fruit.

MATING	ENLARGED OVULES AT 12 DAYS	PLUMP SEEDS AT MATURITY
2n×2n	31.2	26.8
2n×*L. peruvianum*	34.0	.04
4n×4n	7.3	7.9
4n×2n	21.6	.1
4n×*L. peruvianum*	26.3	15.6

gross estimate of the frequency of fertilization may be made by determining the proportion of ovules which increase in size significantly after pollination. Table 3 shows these values for fruits taken at 12 days. The data are based on nine fruits each except in the 2n×2n mating in which ten fruits are represented. On this basis it appears that practically all ovules of 2n plants are fertilized following application of pollen from either 2n *L. pimpinellifolium* or *L. peruvianum*. The sterility associated with the latter mating consequently is attributable to collapse of the hybrid seeds during development. Comparable data for the 2n×4n mating are not available because these fruits all dropped before 12 days.

Infrequent fertilization appears to be an important factor in the low fertility of the 4n×4n mating. Only 7.3 ovules per ovary, on the average, were enlarged at 12 days. On the other hand, a total of 20 seeds and seed-like structures per fruit were found at maturity. The discrepancy is probably due mainly to the elimination of a disproportionate number of fruits with low seeds numbers by abscission. About three times as many ovules become fertile in the 4n×2n mating as in the 4n×4n combination. The very low net fertility of the 4n×2n cross is evidently due to the abortion of seeds during development. The mature fruits resulting from this mating, like those in the 2n×*L. peruvianum* cross, contain no well developed seeds. The frequency of fertilization likewise is fairly high in the 4n×*L. peruvianum* cross. Furthermore, more than half the fertile ovules form plump seeds, in contrast with the 2n×*L. peruvianum* mating in which all or nearly all the hybrid seeds abort.

The occurrence of non-functional seed-like structures within the fruits which mature following the 4n×2n mating agrees with LINDSTROM'S (1932) observations on this cross. LINDSTROM found also that the 2n×4n cross was barren.

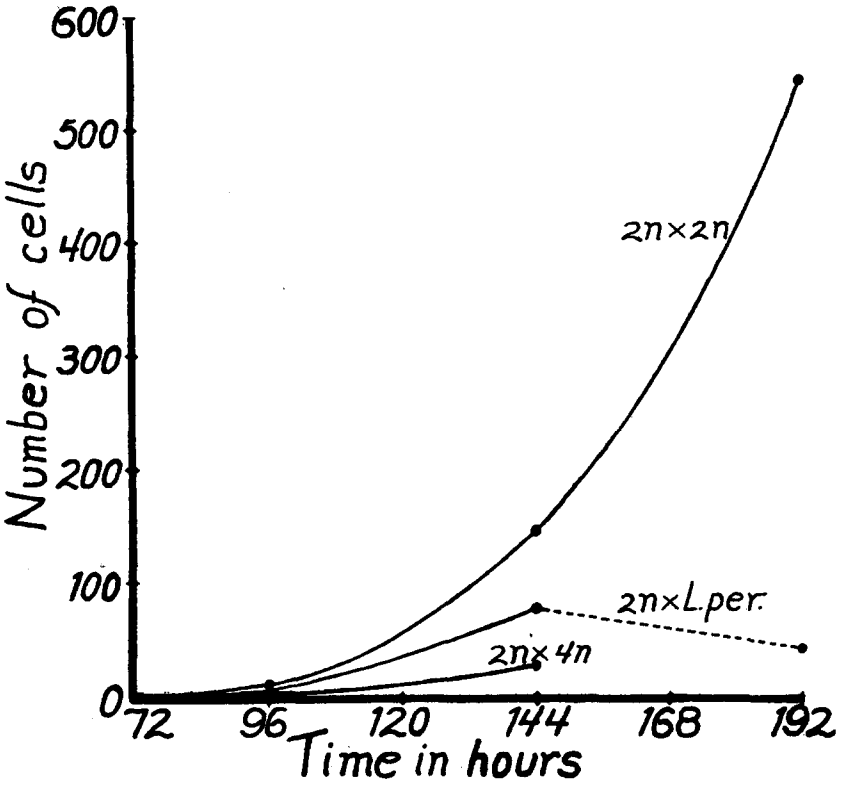


FIGURE 1.—Average number of cells in endosperm following matings involving 2n *L. pimpinellifolium* as the pistillate parent.

RATE OF ENDOSPERM GROWTH

Precocious development of the endosperm is characteristic of the angiosperm seed. *L. pimpinellifolium* conforms to the usual pattern in this respect. Fertilization of the egg and of the central cell are parallel events, but by the time the zygote divides, the endosperm already is a rapidly growing tissue. The ascendancy of the endosperm thus early established is maintained throughout the first stage of seed development. The major phase of embryo growth comes later, although this tissue continues to increase in size steadily, even if slowly, immediately following division of the zygote. Changes affecting the milieu in which the embryo develops occur in the maternal tissues of the seed co-ordinate with, and presumably in response to, the initial activity of the endosperm. It is of primary importance in an analysis of seed collapse, therefore, that early behavior of the endosperm be characterized. Rate of growth of

the tissue at this time is normally high. It appears probable from the results of earlier studies (BRINK and COOPER 1940) and from the tissue relations in *L. pimpinellifolium* to be described below that regular development of other parts of the seed are dependent in a considerable degree upon maintenance of this pace. Accordingly, efforts were made to measure the extent to which rate of endosperm growth varies following the different matings.

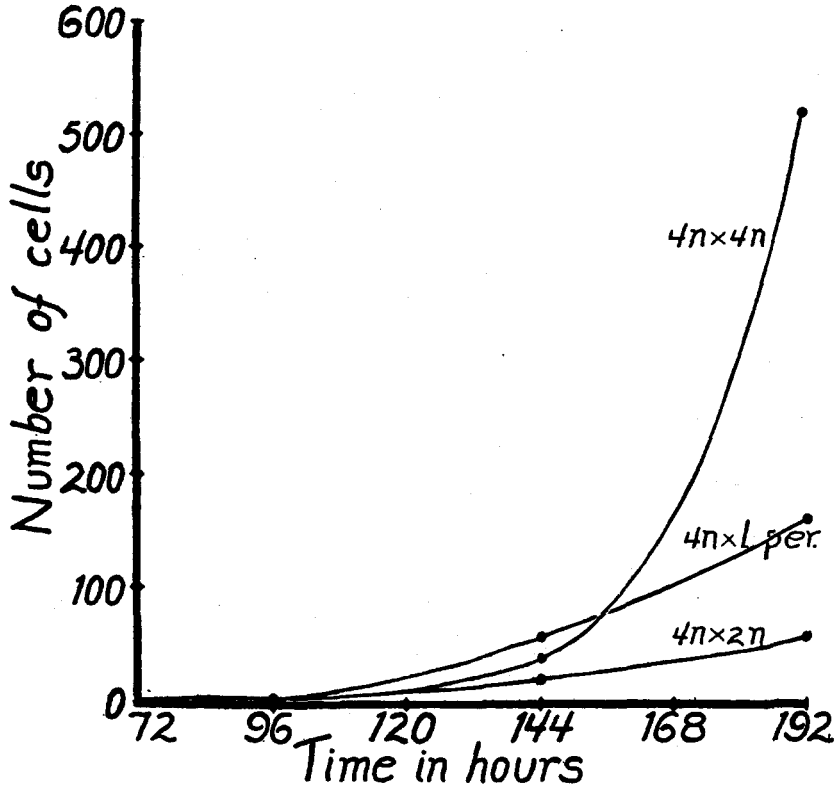


FIGURE 2.—Average number of cells in endosperm following matings involving 4n *L. pimpinellifolium* as the pistillate parent.

Endosperm size, expressed as number of cells, is given in table 4 for the various matings at five periods up to 192 hours after pollination. Cell number was determined from serial sections by counting the nuclei. The endosperm cells are characteristically uninucleate. The 2n×*L. peruvianum* mating at 192 hours, however, is an exception to this rule. Due to a partial breakdown of the tissue, which occurs some time after 144 hours, the parity between nucleus and cell number in the endosperm is destroyed. This unique situation in the 2n×*L. peruvianum* seeds is further described later in the paper.

The data on endosperm size for the matings in which 2n *L. pimpinellifolium* serves as the pistillate parent are plotted in figure 1. It is apparent that the endosperm from the 2n×2n control mating leads the others from the start.

At 144 hours 148 cells are present in the endosperm of the 2n×2n seed, on the average, a value which is twice that for the 2n×*L. peruvianum* endosperm and five times the endosperm size of the 2n×4n seed. The endosperm cell number of the 2n×2n seed increases to an average of 539 at 192 hours. Comparison with the other two classes of seed at this stage cannot be made, because most 2n×4n seeds collapse between 144 and 192 hours, and disintegration of the endosperm begins in the case of the 2n×*L. peruvianum* mating. It is clear from these observations that as compared with normal *L. pimpinellifolium* the

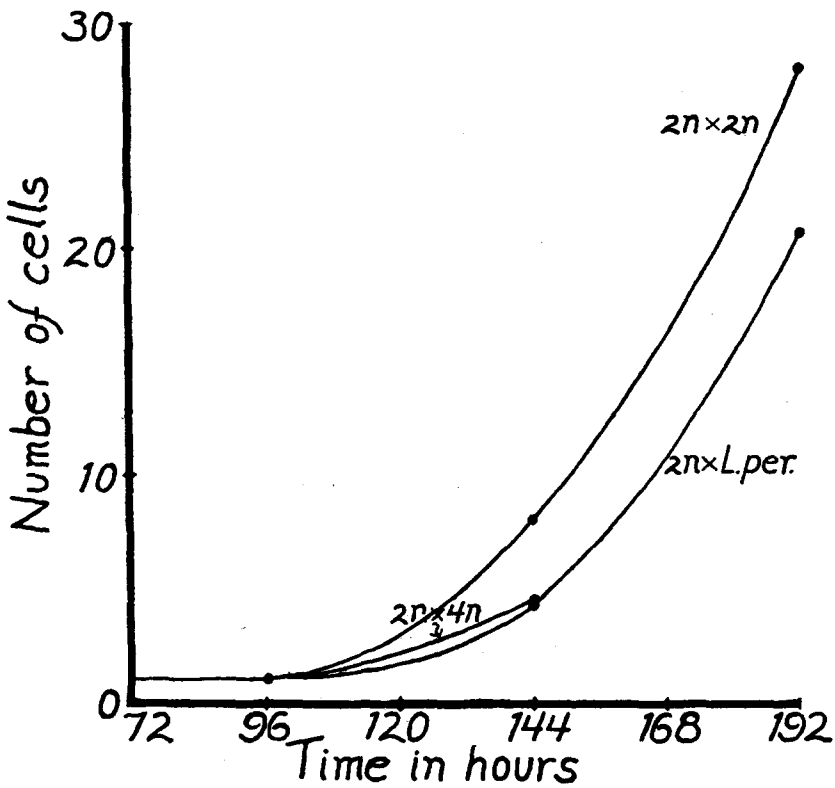


FIGURE 3.—Average number of cells in embryo following matings involving 2n *L. pimpinellifolium* as the pistillate parent.

endosperms formed as a result of the 2n×4n and 2n×*L. peruvianum* matings make a weak growth.

The data from table 4 on endosperm size following the matings in which tetraploid *L. pimpinellifolium* served as the pistillate parent are plotted in figure 2. At 144 hours the endosperm of the 4n×*L. peruvianum* seed is the most advanced averaging 57 cells. The 4n×4n mating is next in order with an average of 36 cells. The endosperms of the 4n×2n seed trail, with a mean of only 20 cells. Endosperm growth in the 4n×4n seeds then accelerates greatly. The tissue was found to have 516 cells at 192 hours, a value which is comparable with that for the 2n×2n seed of the same age. The endosperm of the 4n×*L.*

peruvianum seed continues to grow at an almost steady rate so that at 192 hours the tissue averages 158 cells. It will be recalled from the data presented above that a high proportion of the 4n×4n and 4n×*L. peruvianum* seeds which start to grow are well developed in the mature fruit. The endosperms of the 4n×2n seeds are still very small at 192 hours. Their average size, 55 cells, is about one-tenth the values for the 4n×4n seeds and about one-third that

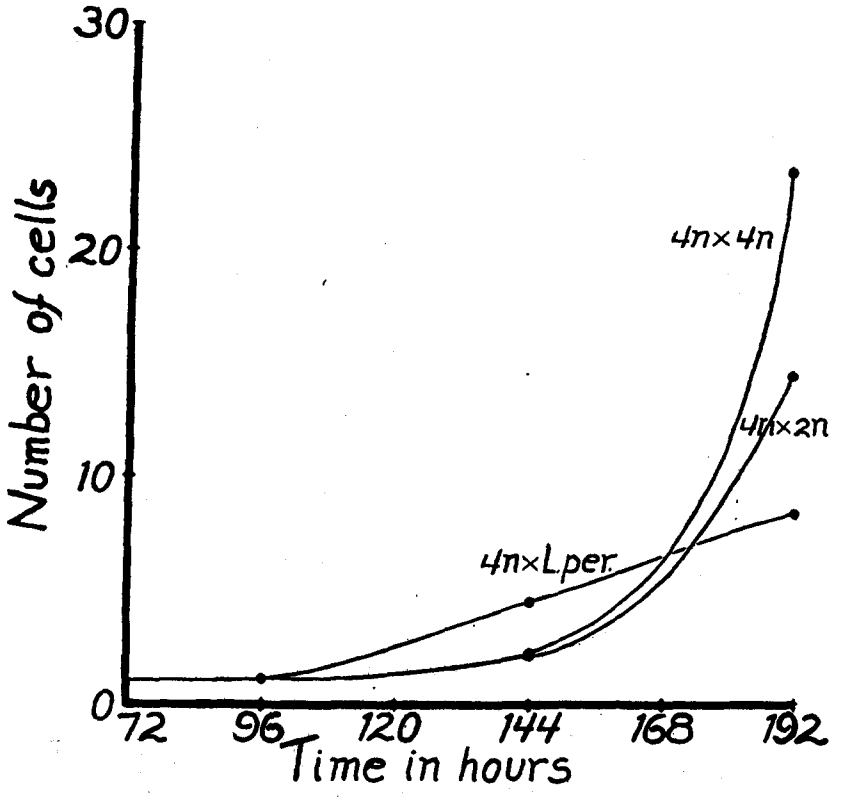


FIGURE 4.—Average number of cells in embryo following matings involving 4n *L. pimpinellifolium* as pistillate parent.

for the 4n×*L. peruvianum* seeds. Unlike these latter two classes, the 4n×2n seeds all collapse before the fruit is ripe.

RATE OF EMBRYO GROWTH

The data presented in table 4 on embryo size for the three matings in which 2n *L. pimpinellifolium* was the pistillate parent are shown graphically in figure 3. It will be observed at once that the numbers of cells in this tissue, particularly at 144 and at 192 hours, are far below those for the corresponding endosperms. Obviously, the growth rate of the embryo at this stage is of a lower order than that of the endosperm.

TABLE 4

Average number of cells in embryo and endosperm at early growth stages after various matings involving 2n and 4n L. pimpinellifolium and 2n L. peruvianum plants.

MATING	NO. OF SEEDS	AVERAGE NO. CELLS	
		EMBRYO	ENDOSPERM
	24 hours		
2n×2n	20	1	1
2n×4n	21	1	1
2n×*L. peruvianum*	14	1	1
4n×4n		not fertilized	
4n×2n		not fertilized	
4n×*L. peruvianum*		not fertilized	
	48 hours		
2n×2n	71	1	1.75
2n×4n	6	1	1
2n×*L. peruvianum*	125	1	1.03
4n×4n	26	1	1.6
4n×2n	138	1	1.2
4n×*L. peruvianum*	50	1	1.06
	96 hours		
2n×2n	200	1	7.8
2n×4n	13	1	4.1
2n×*L. peruvianum*	288	1	3.7
4n×4n	53	1	2.4
4n×2n	306	1	3.5
4n×*L. peruvianum*	191	1	2.3
	144 hours		
2n×2n	25	7.9	148
2n×4n	16	4.4	28
2n×*L. peruvianum*	33	4.2	77
4n×4n	17	2.1	36
4n×2n	56	2.0	20
4n×*L. peruvianum*	30	4.4	57
	192 hours		
2n×2n	4	27.8	539
2n×4n	—	—	—
2n×*L. peruvianum*	21	20.4	(41)*
4n×4n	10	23.4	516
4n×2n	13	14.2	55
4n×*L. peruvianum*	17	8.3	158

* Not comparable; see text.

The most rapidly growing embryos are those in the normal, 2n×2n seeds. These embryos are approximately eight-celled at 144 hours and 28-celled at 192 hours. The embryos of the 2n×*L. peruvianum* and 2n×4n seeds are alike

at 144 hours in being somewhat more than half the size of the controls. The life of the 2n×4n seed is terminated by collapse shortly thereafter. The 2n×*L. peruvianum* seed, however, lives somewhat longer, and the embryo continues to develop, reaching a size at 192 hours about three-quarters of that in the 2n×2n seed.

The data summarized in table 4 on embryo growth in the three classes of seeds resulting from the matings made on tetraploid *L. pimpinellifolium* plants are charted in figure 4. The embryos in the 4n×*L. peruvianum* seeds are the largest at 144 hours, averaging 4.4 cells. The embryos in the 4n×4n and 4n×2n seeds at this time are typically two-celled. This early advantage in embryo size following the outcross to *L. peruvianum*, however, is not maintained. At 192 hours the embryos in the 4n×4n seeds are the largest, approaching in fact the size of those in the normal diploid seeds in the (a) series of matings. The 4n×2n seeds possess more advanced embryos than do the 4n×*L. peruvianum* seeds at 192 hours, the average in the latter case being only 8.3 cells. The non-correspondence between size of young embryo and seed survival in the (b) series of matings is evident. Both the 4n×4n and 4n×*L. peruvianum* seeds in relatively high proportion are capable of developing to maturity, whereas the 4n×2n seeds regularly fail. The latter class of seeds, nevertheless, occupies an intermediate position at 192 hours with reference to embryo size.

ENDOSPERM DEVELOPMENT IN RELATION TO SIZE OF THE ASSOCIATED EMBRYO

Size of endosperm in the different classes of seeds has been considered above in relation to time elapsed after pollination. The data on 144-hour seeds are summarized in table 5 so as to show cell number of the endosperm in relation to size of the associated embryo.

It will be observed from table 5 that, within a given mating, average number of cells in the endosperm increases as the embryo size rises. That is to say, there is a well marked positive correlation in rate of growth of these two tissues in all the classes of seeds.

The effects on endosperm cell number, embryo size remaining constant, of varying the type of staminate parent in matings with diploid and tetraploid *L. pimpinellifolium*, respectively, are of particular interest. Several comparisons are possible in the series of matings in which 2n *L. pimpinellifolium* was used as the pistillate parent. Seeds of this group containing four-celled embryos, for example, average 105 endosperm cells in the 2n×2n mating, 31 in the 2n×4n cross, and 79 in the 2n×*L. peruvianum* mating. The rank is the same for seeds with embryos of the other sizes which are represented in the three different matings. This means that embryos of a given size in the two classes of seeds which collapse during development, 2n×4n and 2n×*L. peruvianum*, are accompanied by smaller endosperms than are embryos in the control 2n×2n seeds which mature normally.

A similar relation is evident in the data relating to the matings in which 4n *L. pimpinellifolium* was the pistillate parent. The seeds from the 4n×2n

cross alone in this series regularly fail, and it is in this mating that the smallest endosperms for a given size of embryo are found.

TABLE 5

Number of endosperm cells associated with embryos of given cell numbers at 144 hours after pollination.

(a) 2n as pistillate parent

NO. CELLS IN EMBRYO	2n×2n		2n×4n		2n×*L. peruvianum*	
	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM
1	—	—	2	15	—	—
2	—	—	3	13	2	45
3	—	—	1	15	3	39
4	1	105	3	31	20	79
5	—	—	2	30	4	91
6	3	140	1	13	3	101
7	7	141	2	41	1	100
8	4	144	1	31	—	—
9	6	162	—	—	—	—
10	4	159	1	80	—	—
Total and average	25	148	16	28	33	77

(b) 4n as pistillate parent

NO. CELLS IN EMBRYO	4n×4n		4n×2n		4n×*L. peruvianum*	
	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM	NO. OF SEEDS	AVGE. NO. CELLS IN ENDOSPERM
1	—	—	7	14	—	—
2	16	35	39	21	2	32
3	1	48	10	23	—	—
4	—	—	—	—	13	49
5	—	—	—	—	11	63
6	—	—	—	—	4	77
Total and average	17	36	56	20	30	57

EARLY GROWTH OF THE SEED

The stimulus to growth arising from double fertilization is not restricted to the egg and the central cell within the embryo sac but extends quickly to the contiguous maternal parts of the ovule as well. The following data, based on five seeds in each case, illustrate the large increase in size of ovule which occurs between the mature embryo sac stage and 144 hours after pollination. The values were obtained by measuring the thickness of seeds which had been so

cut on the microtome that the median section passed through both embryo (or egg) and chalazal pocket.

MATING	DIAMETER IN MICRONS	
	MATURE OVULE	144-HOUR SEED
2n×2n	132	271
2n×*L. peruvianum*	132	254
2n×4n	132	211
4n×4n	154	262
4n×*L. peruvianum*	154	272
4n×2n	154	226

The above measurements make it clear that endosperm and embryo do not begin development in a quiescent medium but are required to establish themselves within a relatively large mass of maternal tissue which is simultaneously aroused to rapid expansion.

We have shown in earlier publications how an unbalance of growth between endosperm and integument following enforced self-fertilization in *Medicago sativa* (BRINK and COOPER 1940) and after interspecific hybridization in Nicotiana (COOPER and BRINK 1940) may lead to breakdown of the seed. Since the evidence in these cases suggested that the unbalance might be widely operative in causing seed collapse, close attention was given to the relation between endosperm and maternal tissue in the seeds resulting from the present matings.

TABLE 6

Thickness in microns of the endosperm and endothelium of seeds at 144 and 192 hours.

MATING	THICKNESS IN MICRONS				RATIO ENDOSPERM:ENDOTHELIUM	
	144 HOURS		192 HOURS			
	ENDOSPERM	ENDOTHELIUM	ENDOSPERM	ENDOTHELIUM	144 HRS.	192 HRS.
2n×2n	103	24	156	24	4.3	6.5
2n×*L. peruvianum*	58	41	58	48	1.4	1.2
2n×4n	50	24	—	—	2.1	—
4n×4n	84	24	161	24	3.6	6.7
4n×*L. peruvianum*	86	24	120	24	3.6	5.0
4n×2n	50	34	55	43	1.5	1.3

The data in table 6 show the average thickness of endosperm and endothelium in the six classes of seeds at 144 and 192 hours. Five seeds from each mating in which the median section passed through embryo and chalazal pocket were chosen for measurement. The thickness was computed from the number of 12-micron sections in which the respective tissues were represented in passing across the seed along the plane predetermined as stated. Data on

volume rather than thickness would reveal more accurately the size relations of the two tissues, but the added information thus afforded was not deemed of sufficient importance to justify the greatly increased work involved in determining the third dimension.

Although the data are crude, they clearly reveal a large difference between the viable and inviable classes of seeds at these early stages in relative growth of endosperm and endothelium. This relation is brought out in the last two columns of the table showing the ratio in thickness of the two tissues. The viable classes of seed, 2n×2n, 4n×4n, and 4n×*L. peruvianum*, have large endosperms and uniformly thin endotheliums and, hence, high ratios. The seeds destined, on the other hand, to collapse, 2n×*L. peruvianum*, 2n×4n, and 4n×2n, have smaller endosperms and (with the exception of 2n×4n at 144 hours) thicker endotheliums, with resulting low endosperm-endothelium ratios.

More detailed observations on endosperm and endothelium in the six classes of seeds are presented in the section following.

THE 2n×2n SEED

Fertilization occurs in diploid *L. pimpinellifolium* within 24 hours after pollination. Evidence of the rapid endosperm development following fertilization has been presented in table 4 and figure 1, above. The early changes appearing in the maternal tissues of the seed are illustrated in figure 6, based on 144-hour material. Figure 6 may be compared with figure 5 showing the ovule in median section at the mature embryo sac stage. A large increase in size of the developing endosperm and embryo which arise from the fertile embryo sac and in size of the entire seed occurs during the first six days. Enlargement of the integument results both from cell expansion and increase in cell number by mitotic division. The cells lying between the apex of the single vascular bundle entering the seed and the chalazal pocket become more clearly differentiated into a conducting tissue thus providing a direct nutritive connection between the endosperm and regions outside the seed.

The apical and lateral cells of the nucellus become disorganized and ultimately disintegrate during the course of development of the ovule so that the mature embryo sac is immediately surrounded by the massive integument. Following fertilization the inner epidermis of the integument, the endothelium, becomes differentiated into a well-defined layer of densely-cytoplasmic cells. This endothelium is continuous with the lining of the micropylar canal and surrounds the expanding endosperm except for a small gap at the chalazal end where it assumes a tube-like form leading toward the vascular bundle. It is opposite the gap that the small chalazal pocket in which the conducting tissue terminates is formed before fertilization occurs. The endothelium normally remains one cell in thickness, the cells dividing along the radial axis as the seed grows.

A few to several layers of integumentary cells immediately outside the endothelium lose their cytoplasmic contents during early development of the seed

(fig. 6, 7). The matrix of cell walls persists so that the shape and position of the inner portions of the seed are not altered. The depleted tissue becomes nearly continuous around the endothelium by 192 hours. The distal cells of the conducting strand tend to remain nucleated and retain at least a part of their cytoplasmic contents. The depletion is somewhat less complete, therefore, in the chalazal region than elsewhere.

It is probable that the protoplasts in the depleted part of the living seed are replaced with a liquid which fills the interstices of the matrix. This would mean that the sac-like endothelium enclosing endosperm and embryo is supported in a relatively homogeneous fluid medium, the homogeneity being maintained by the ready diffusion of solutes in the absence of semipermeable membranes.

The integumentary cells which become emptied of their contents serve as an initial source of nutriment for the young endosperm, embryo, and associated endothelium. The main source of food, however, is outside the seed. Access to it is afforded by the vascular bundle. The movement of nutrients into the endosperm appears to take place in part through the chalazal aperture lying opposite the end of the conducting tissue and in part also through the endothelium. It will be seen from figure 6 that the cytoplasm of the endosperm cells opposite the chalazal pocket is relatively dense, a condition suggesting an active rôle in the absorption of nutrients. Frequently the endosperm cells in this region are seen to be elongated in the direction in which nutrients would be expected to move. The peripheral cells of the endosperm, in general, are somewhat more densely cytoplasmic than those of the interior. It is suggested that this condition is associated with the absorption of nutrients from the endothelium which appears to be active in promoting cytolysis in the adjacent integumentary tissue. Food materials entering the seed through the vascular bundle and diffusing into the depleted portion of the integument, likewise, may be absorbed by the endosperm through the endothelium.

THE 2n×L. PERUVIANUM SEED

The frequency of fertilization in the 2n×*L. peruvianum* cross is as high as that in the 2n×2n mating, but all the seeds collapse before the fruit is ripe. The changes leading to failure are of interest in themselves but were studied in the present case principally for the purpose of relating them to the events which terminate in breakdown of the 2n×4n seed.

Growth of the endosperm is retarded compared with that of normal *L. pimpinellifolium*. This fact is evident from the data presented in table 4 and figure 1. Embryo growth in the 2n×*L. peruvianum* seed is slowed down also, but the relative decrease is less than that in the endosperm.

The cells of the endosperm immediately adjacent to the embryo at 144 hours have dense cytoplasm, whereas those at the chalazal end are highly vacuolate and appear starved (fig. 10). As growth continues, the endosperm increases considerably in volume in spite of the fact that there is a decrease in the number of cells. All the cells are large and highly vacuolate at 192 hours (fig. 11,

12). A few of the cells on the dorsal side of the endosperm near the apex of the embryo have become greatly increased in size due to the breakdown of intervening cell walls and the fusion of two or more cells, which accounts for the decrease in cell number. The nuclei of these fusing cells likewise unite so that large nuclei with numerous nucleoli are present. Further enlargement of the cells and continued fusion of those cells in the micropylar region takes place so that by 288 hours the embryo comes to lie in a single enormous cell which occupies the micropylar half of the endosperm, as shown in figure 13. A giant nucleus is present. Continuing dissolution of cell walls and fusion of nuclei is evident at the chalazal end of this cell. This type of breakdown persists until the entire endosperm is comprised of only a very few cells. The endosperm then collapses, and shortly thereafter growth of the embryo and seed ceases.

The effects of outcrossing diploid *L. pimpinellifolium* to *L. peruvianum* on early behavior of the accessory tissues of the seed are illustrated in figures 10 and 11. A conspicuous thickening of the endothelium mainly on the dorsal side of the endosperm near the chalazal pocket is evident at 144 hours (fig. 10). The cells in this region are becoming meristematic. The starved condition of the proximal portion of the endosperm adjacent to the hypertrophied endothelial tissue is significant. Noteworthy also is the accumulation of deeply staining particles in the chalazal pocket. The nature of this material has not been determined. Depletion of the integumentary cells adjacent to the endothelium proceeds at about the same rate as in 2n×2n seeds.

THE 2n×4n SEED

Endosperm development is slow from the start in the short-lived 2n×4n seeds. The data in table 4 show that the tissue is only about one-half the size of the control endosperm at 96 hours and at 144 hours has only 28 cells, on the average, in comparison with 148 cells for the normal endosperm from 2n×2n matings. Growth of the embryo is retarded also.

A marked hypertrophy of the endothelial cells lying along that portion of the dorsal surface of the endosperm nearest the chalaza is clearly in evidence at 144 hours (fig. 16). Depletion of the cells in the inner portion of the integument does not proceed as rapidly as in the 2n×2n seed. As in the 2n×*L. peruvianum* seed, there is a considerable accumulation of stainable material just outside the chalazal aperture.

THE 4n×4n SEED

The developing seed of the tetraploid resembles that of the diploid very closely (fig. 8, 14). Fertilization, which occurs within 24 hours after pollination of the 2n plant, takes place somewhat later in the tetraploid. Growth of the endosperm also starts a little more slowly. Cell number in this tissue, however, is increasing very rapidly at 192 hours, and at this time the size of the tissue is nearly equal to that of the diploid. The embryo of the tetraploid follows a course of growth parallel to that of the endosperm in that it starts slowly but approaches the size of the diploid embryo at 192 hours.

Through differentiation of the intervening integumentary cells the endosperm of the tetraploid early establishes through the chalazal pocket a direct nutritive connection with the vascular bundle of the seed. The extent and rate of the differentiation appears to be slightly greater after the 2n×2n and 4n×4n matings than in the other classes of seeds.

The endosperm cells containing the densest cytoplasm are situated opposite the chalazal aperture and on the surface of the endosperm in contact with the endothelium (fig. 9). The endothelium remains a single layer of cells in thickness as the seed grows; and the cells of the inner portion of the integument gradually lose their contents. The 4n×4n seed is indistinguishable from that of the diploid in these respects.

THE 4n×L. PERUVIANUM SEED

About 60 percent of the ovules which become fertile in the 4n×*L. peruvianum* mating develop into plump seeds. This relatively high net fertility stands in sharp contrast with the early and complete abortion of seeds following the 2n×*L. peruvianum* cross.

The endosperm of the 4n×*L. peruvianum* seed grows more slowly than that of the 4n×4n seed so that at 192 hours it is only about one-third as large (table 4). The regions of densest cytoplasm in the endosperm are opposite the chalazal pocket and adjacent to the endothelium (fig. 15). No evidence of nuclear fusion, such as occurs in the endosperm of the 2n×*L. peruvianum* seed, has been observed in this tissue. The embryo of the 4n×*L. peruvianum* seed is about one-half the size of that in the 4n×4n seed at 192 hours.

The condition of the accessory tissues of the 4n×*L. peruvianum* seed at 144 hours is illustrated in figure 15. The endothelium consists of a single cell layer, although there is a tendency in some seeds for the cells to thicken in the dorsal region near the chalazal pocket. Rarely, however, do these cells divide in the plane normal to the endothelium. The depleted area of the integument is similar in extent to that in the 4n×4n seed.

THE 4n×2n SEED

The tetraploid used as the pistillate parent in matings with the diploid gives a high proportion of fertilized ovules, but the seeds are incapable of developing to a germinable condition.

Growth of the endosperm in the 4n×2n seed is conspicuously weak. The tissue possesses only 55 cells, on the average, at 192 hours (table 4). This is about one-third the number in the 4n×*L. peruvianum* seed and approximately one-ninth as many as occur in the 4n×4n seed at this time. The embryo resulting from the 4n×2n mating is comparable to that of the 4n×4n seed up to 144 hours, but it falls sharply behind at 192 hours. The embryo of the 4n×2n seed at 192 hours, however, is larger than that of the 4n×*L. peruvianum* seed.

Differentiation of the tissue between the apex of the vascular bundle and the chalazal pocket is less complete at 144 hours than in the 4n×4n seed.

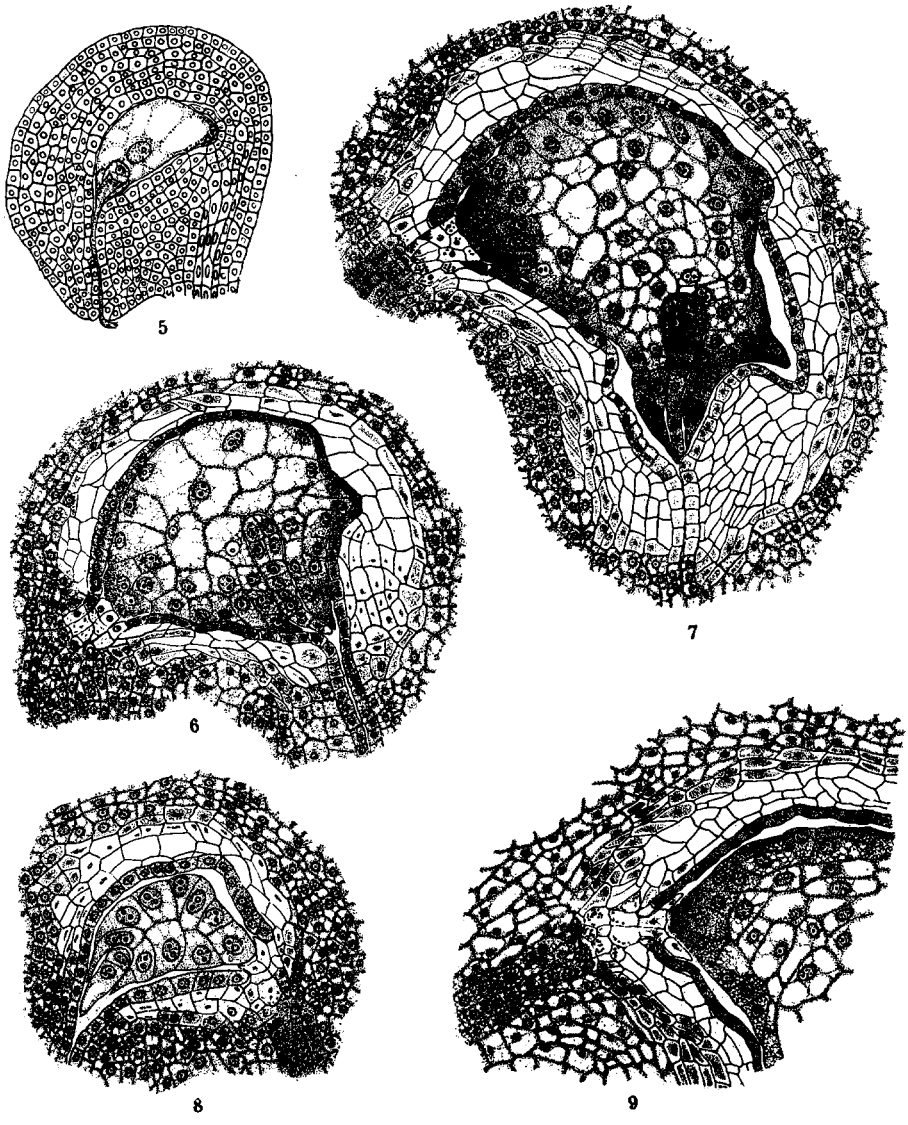


FIGURE 5 TO 7.—2n×2n.—Fig. 5. Ovule at time of fertilization.—Fig. 6 and 7. Endosperm and adjacent tissue at 144 hours and 192 hours, respectively, following pollination.

FIGURE 8, AND 9.—4n×4n.—Fig. 8. Endosperm and adjacent tissue at 144 hrs.—Fig. 9. Chalazal pocket region at 192 hours. ×132. Note the single layer of densely cytoplasmic endothelial cells. Several layers of integumentary cells immediately adjacent thereto lose their contents in the course of development. Compare fig. 6 and 7.

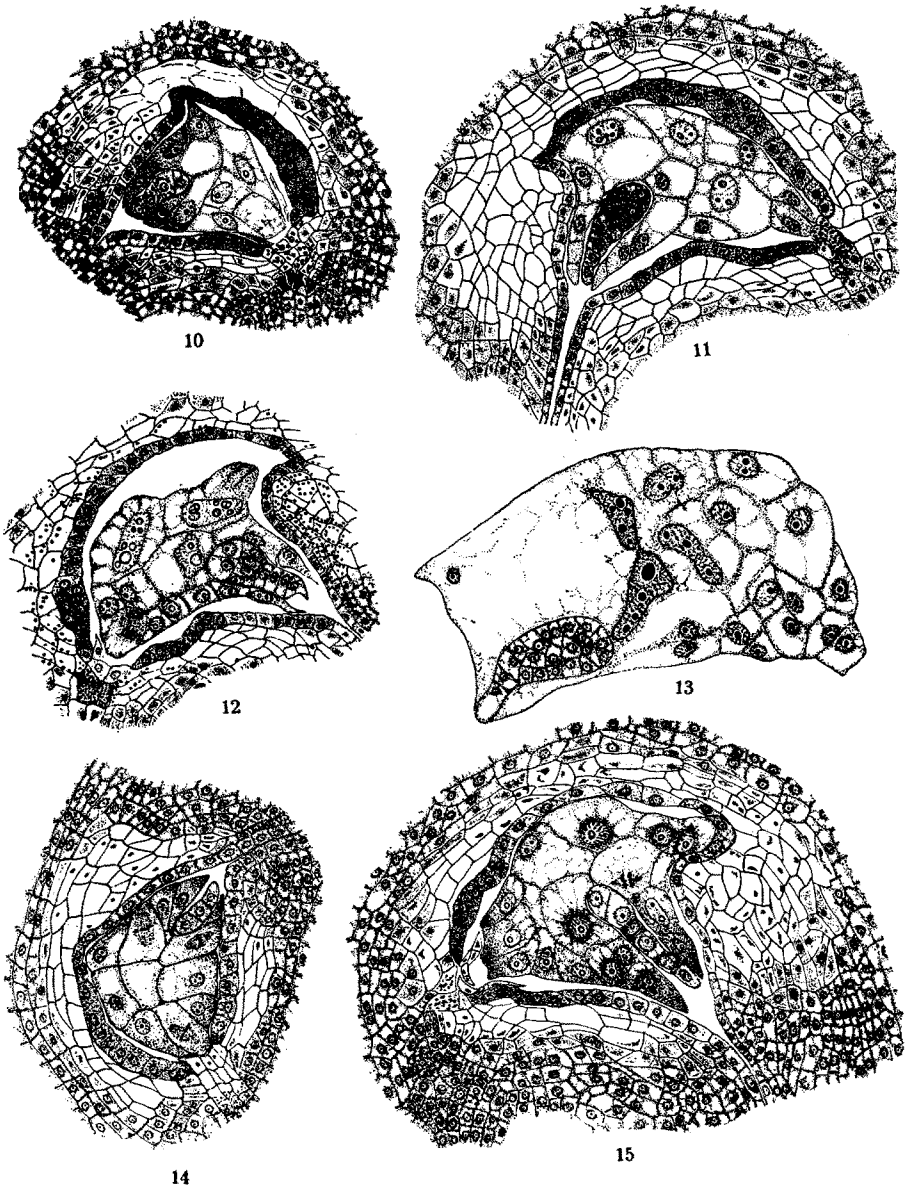
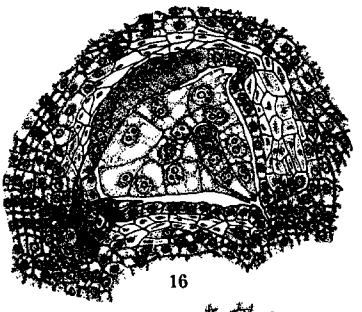


FIGURE 10 TO 15.—2n×*L. peruvianum*.—Fig. 10. Endosperm and immediately adjacent tissue at 144 hours showing starved condition of chalazal cells of endosperm. The endothelial cells on the dorsal surface of the endosperm are greatly increased in size.—Fig. 11 and 12. Same at 192 hours. Some cells of the endosperm contain giant nuclei. The cells of the chalazal pocket are filled with a densely staining material.—Fig. 13. Endosperm and embryo at 288 hours showing the breakdown of those endosperm cells in the region of the embryo and the fusing nuclei. ×132.—FIG. 14 AND 15.—Endosperm and adjacent portion of integument of 144 hours in 4n×4n and 4n×*L. peruvianum*, respectively. ×132.



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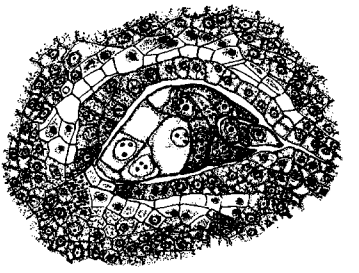

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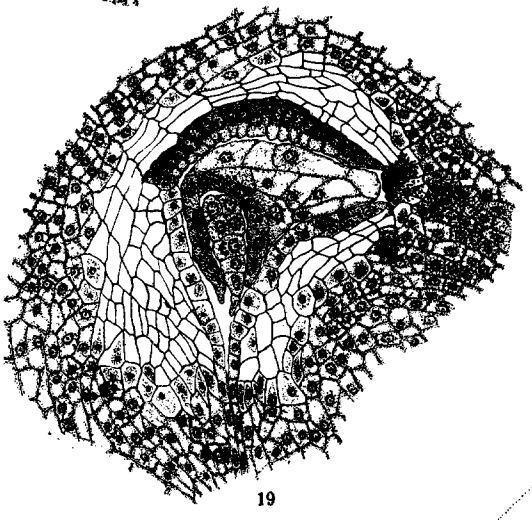

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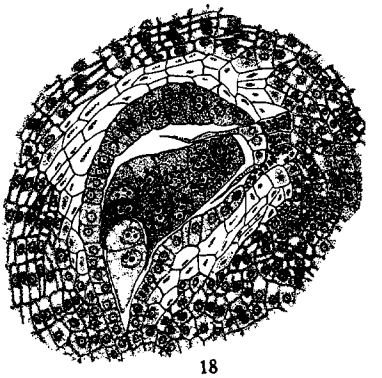

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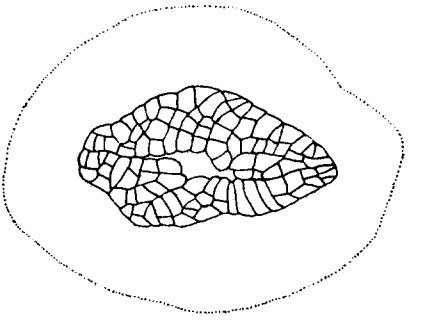

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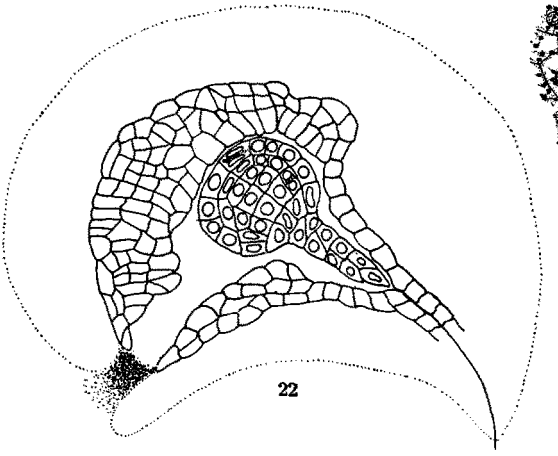
FIGURE 16 TO 19. Endosperm, endothelium, and immediately adjacent cells of integument. ×132.—Fig. 16. 2n×4n, at 144 hours. Note highly vacuolate cells of endosperm and enlarged endothelial cells.—Fig. 17 and 18. 4n×2n at 144 hours showing lag in development of the endosperm and the overgrowth of the cells of the endothelium.—Fig. 19. Same at 192 hours showing meristematic condition of the cells of the endothelium. The cells of the chalazal pocket are filled with a granular material.

FIGURE 20.—4n×2n. Polar view of mitotic figure in the endosperm showing 60 chromosomes. ×1320.

FIGURE 21.—4n×2n. Transverse section through overgrown endothelium at 288 hours. Note small endosperm cavity. The dotted line shows the limits of the cells of the integument which are empty. ×132.



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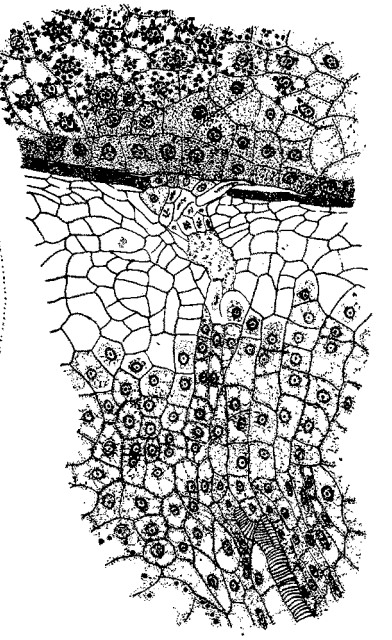

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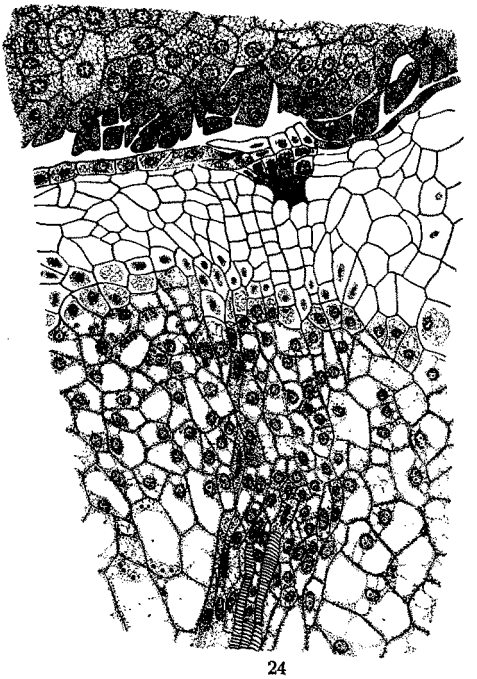

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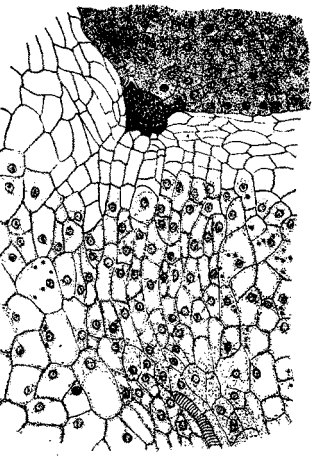

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FIGURE 22.—4n×2n. Embryo and overgrown endothelium at 288 hours. Note the rounded embryo, empty endosperm cavity and densely staining material in chalazal region opposite endosperm cavity. ×132.

FIGURE 23 TO 25.—Longitudinal sections of chalazal portions of seeds at 384 hours showing relationships between endosperm, endothelium, and vascular tissue.—Fig. 23. 4n×4n showing densely cytoplasmic absorbing cells of the endosperm immediately opposite chalazal pocket. Starch is being stored in the highly vacuolate central cells of the endosperm.—Fig. 24. 4n×*L. peruvianum*. The epidermal cells of the endosperm are breaking apart, and no starch is being stored in the central densely cytoplasmic cells. The endothelium persists as a single layer of cells.—Fig. 25. 4n×2n showing extensive hyperplasia of the endothelium. Note that in figures 24 and 25 the cells of the chalazal pocket are packed with a granular material. ×132.

Deeply staining granules accumulate in the chalazal pocket of the 4n×2n seed and are frequently seen also within the endosperm at 144 hours (fig. 17, 18). If these particles are reserve food of some sort, they nevertheless do not insure against starvation of the endosperm. The chalazal portion of the endosperm may become depleted nearly to the point of collapse in spite of an abundance of the granules in nearby cells (fig. 17). Evidently some substances other than those in the deeply staining granules are in short supply in the starving tissue.

Hyperplasia of the endothelium in the 4n×2n seed is apparent at 144 hours (fig. 17, 18). Proliferation of the cells begins near the chalaza in the dorsal region of the endothelium. Figure 19, based on a 192-hour seed, shows the hyperplastic portion of the endothelium extending dorsally from the chalaza to a point opposite the anterior end of the embryo. The cells of the endosperm adjacent to the overgrown portion of the endothelium are very poor in cytoplasm. The endosperm has broken down entirely by 288 hours, and the embryo is surrounded by a mass of overgrown endothelial tissue, as illustrated in figures 21 an 22.

THE SEVERAL CLASSES OF SEEDS AT 16 DAYS

The 2n×2n mating and two of the matings on tetraploid plants, 4n×4n and 4n×*L. peruvianum*, yield a high proportion of viable seeds. A few seeds from the 2n×*L. peruvianum* and 4n×2n crosses persist for 16 days, although this is about the limit of their life span. It is of interest to examine the histological condition of these seeds at the time of their ultimate separation into two classes on the basis of viability.

The relation of the tissues in the chalazal region of the 4n×4n seed is depicted in figure 23. Considerable storage of granular food reserves in the endosperm has occurred. The outer layer of endosperm cells, and the cells in the region opposite the chalazal aperture, however, are densely cytoplasmic and free of visible storage materials. Apparently the main function of these cells is absorption of nutrients from the solution with which the cell wall matrix surrounding the endosperm presumably is filled. It will be noted from figure 23 that the conducting tissue distal to the vascular bundle is well organized in the form of rather regular columns of cells leading toward the chalazal pocket region.

The most conspicuous differences in the 4n×*L. peruvianum* seed at this time, as illustrated in figure 24, appear in the endosperm. In the first place no granular reserves are found in this tissue, which is rather densely cytoplasmic in the entire outer portion. Secondly, the endosperm cells adjacent to the endothelium are separating from each other. This partial disorganization may result from growth of the endothelium at the expense of the endosperm protoplasts in the absence of food reserves. There is a considerable accumulation of deeply staining material in the chalazal pocket. The conducting tissue at the apex of the vascular bundle is somewhat less clearly differentiated from the surrounding cells than is the case in the 4n×4n seed.

The endosperm of the 4n×2n seed has disappeared entirely by 16 days, and the space which it normally occupies has become nearly filled with a densely staining mass of overgrown endothelial tissue (fig. 25). The material of unidentified character which begins to accumulate early in the chalazal pocket in seeds of this class is present in large amounts at this stage. The conducting tissue is similar in appearance to that of the 4n×*L. peruvianum* seed.

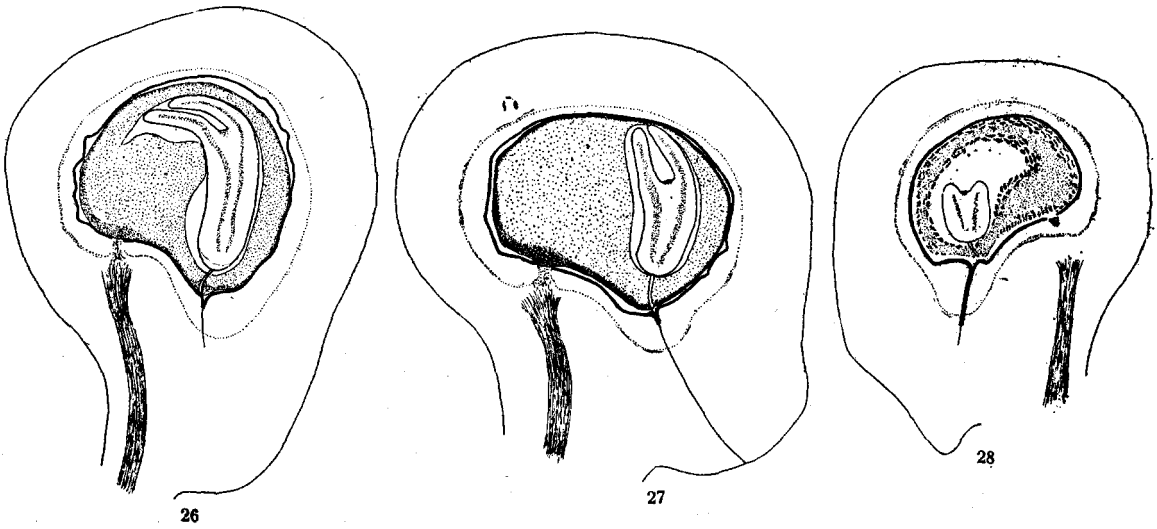

FIGURE 26 TO 28.—Median longitudinal sections through developing seeds at 384 hours showing the relationship existing between embryo, endosperm, integument, and vascular tissue. ×22. The dotted line outside the endothelium shows the limits of the empty cells of the integument (semi-diagrammatic).—Fig. 26. 2n×2n. The embryo with well developed cotyledons grows at the expense of the endosperm. The principal absorbing region of the endosperm is immediately opposite the chalazal pocket.—Fig. 27. 4n×4n. The development is similar to that found in the 2n×2n seed.—Fig. 28. 4n×*L. peruvianum*. Development is much slower than in the 4n×4n seed. The small embryo is differentiating in a normal manner. The peripheral layer of endosperm cells and those adjacent to the embryo are breaking down.

An outline drawing of a median section of the 2n×2n seed at 16 days is shown in figure 26. The embryo, imbedded in the endosperm, shows prominent cotyledons and a well defined vascular tissue. It is apparent from figure 27 that the 4n×4n seed of the same age is closely comparable. Gross seed size is not greatly different, the extent of depleted tissue, the boundary of which is indicated by a dotted line, is about the same, and in both seeds the endothelium is a thin and relatively inconspicuous tissue. The embryo of the 4n×4n seed is slightly smaller than that of the diploid at this stage.

The 4n×*L. peruvianum* seed at this time is definitely smaller than its 4n×4n counterpart (fig. 28). As shown in detail in figure 24, the outer cells of the endosperm are becoming separated from each other, and this tissue is being digested in the boundary zone of the relatively large cavity in which the embryo lies. The embryo is much less advanced than that in the 4n×4n

seed, although normal in form. The data in table 2 show that about one-half the fertile ovules from the 4n×*L. peruvianum* mating develop into plump seeds. Figure 28 is illustrative of the best developed seeds of this class at 16 days.

DISCUSSION

The results, in terms of seed production, of the four *L. pimpinellifolium* matings reported upon here agree with the findings of other investigators in showing that autotetraploids are fertile *inter se*, like diploids, but that reciprocal crosses between tetraploids and diploids are sterile, or nearly so. The tetraploid tomato produces considerably fewer seeds than the diploid, but this is due mainly to failure of many ovules to become fertile because of restricted pollen tube growth rather than to breakdown of seeds during development.

The course of seed development following the 4n×4n mating agrees in detail with that of diploid *L. pimpinellifolium*. The endosperm of the tetraploid starts growth more slowly, but by eight days it is on a par with that of the diploid. A similar initial lag characterizes the tetraploid embryo also. The endothelium, a single layer of specialized cells investing the endosperm, behaves regularly in the 4n×4n seeds as does the adjacent integumentary tissue, which becomes depleted of its protoplasmic contents. The relationship established between the vascular bundle entering the seed and the chalazal pocket, which lies opposite a small aperture in the endothelium, by differentiation of the intervening cells into a conducting tissue, is essentially the same in the tetraploid as in the diploid seed. In kind and sequence alike the histological changes involved in transformation of the fertile ovule of the tetraploid into a normally developed seed are indistinguishable from those in the diploid.

When, however, the diploid is substituted for the tetraploid as staminate parent in the mating with the tetraploid or, *vice versa*, the tetraploid is used as staminate parent in crosses with the diploid, the behavior of the resulting fertile ovules is quite different. Both the 4n×2n and 2n×4n seeds fail to develop to a germinable condition. Significant also is the fact that the course of events leading to collapse, so far as it can be discerned histologically, is the same in the two kinds of seeds. The first detectable departure from normal behavior, as exemplified by the respective 4n and 2n controls, is a decreased rate of endosperm growth. Table 4 shows that at the three periods beyond fertilization at which observations were made—namely, 48, 96, and 144 hours—the endosperm of the 2n×4n seed contains fewer cells than the endosperm of the 2n×2n seed. The difference in endosperm size increases with age of the seed, becoming five-fold at 144 hours. Although the 96-hour seeds do not conform, the same general relation holds in comparing 4n×2n and 4n×4n seeds. The endosperm of the latter is nearly twice the size of that of the former at 144 hours and over four times the size at 192 hours. It is evident from table 5 also that for a given size of embryo the number of endosperm cells is regularly greater for 2n×2n than for 2n×4n seeds and for 4n×4n than for 4n×2n seeds.

The endothelium in 2n×4n and 4n×2n seeds departs at an early stage from the course of development followed by the diploid and the tetraploid. The difference is discernible in some seeds at 96 hours and becomes pronounced by 144 hours. The endothelium in the control seeds comprises a single layer of cells flattened against the endosperm which it surrounds. Growth of the endothelium by cell division is coordinate with that of the enlarging endosperm, the latter being the more aggressive tissue. It would appear that in the normal seed the size of the endothelium is merely accommodated to that of the rapidly expanding endosperm, to which it is subordinate. This nicely adjusted balance is upset very early in 2n×4n and 4n×2n seeds. The sequence of abnormal developmental changes which then follows leads directly, even if not always quickly, to collapse of the entire seed.

The first visible evidence of atypical growth of the endothelium is a thickening of the cells in the dorsal region near, but not immediately adjacent to, the chalazal pocket. As these cells become increasingly meristematic in appearance they orientate themselves with their long axes vertical to the endosperm surface. The long axis thus comes to lie at right angles to the normal position. Clear evidence of polarization of the cells in this direction is sometimes seen (fig. 19). The endosperm cells just beneath the over-active part of the endothelium become thinly cytoplasmic and starved in appearance. The evidence afforded by these tomato seeds leaves no room for doubt that weak growth of the endosperm and hyperplasia of the endothelium are intimately related phenomena.

Overgrowth of the endothelium continues and is accompanied by further impairment of the endosperm. Eventually the endosperm is entirely destroyed. The embryo, already much retarded in growth and obviously undernourished, although not yet beginning to disintegrate, now lies in a small cavity surrounded by a large mass of activity proliferating endothelial tissue. The embryo may continue to live for several days under these conditions. Growth soon ceases, however, and the entire seed collapses.

The course of developmental events leading to breakdown of the 2n×4n and 4n×2n *L. pimpinellifolium* seeds parallels in all essential features that which the writers have described for certain interspecific hybrid seeds in Nicotiana (COOPER and BRINK 1940). Weak growth of the endosperm and overgrowth of the adjacent maternal tissue characterize the seeds which fail in both these cases. The embryo dies not because it is inherently defective, but as a result of disturbances in the accessory tissues of the seed which render the latter an unfavorable medium for continued growth of the embryo.

The close correspondence between seed failure associated with species hybridization and with crosses between diploids and tetraploids of the same species is further attested by the behavior of the 2n *L. pimpinellifolium*×*L. peruvianum* mating, as described above. The endosperm of the young seeds from the tomato species cross grows slowly like that resulting from the 2n×4n mating, and the endothelium becomes hyperplastic in the same way and at the same time. SANSOME, SATINA, and BLAKESLEE (1942) have observed that the

inviable seeds of *Datura stramonium*×*D. metel* and *D. stramonium*×*D. ceratocaula* likewise behave much as those from the 4n×2n mating in *D. stramonium*.

The breakdown of endosperm cell walls accompanied by the formation of giant nuclei in the vicinity of the embryo observed at 192 hours after the 2n×*L. peruvianum* mating, however, does not have a counterpart in the 2n×4n seeds. Disintegration of the endosperm in the vicinity of the embryo appears to be a premature manifestation of the parasitism which is normal to the relationship between these tissues. The early onset of cellular dissolution in this region indicates an incapacity of the crossbred endosperm to maintain its function as a nurse tissue in association with an embryo whose absorptive power is not correspondingly impaired by the hybrid condition. This endosperm-embryo imbalance doubtless hastens death of the 2n×*L. peruvianum* seed. It is not, however, the primary factor in causing it. This is shown by the fact that the hyperplastic condition of the endothelium which, in association with a weak endosperm, regularly leads to collapse has already become established in the seed before any evidence of cellular breakdown in the vicinity of the embryo appears. The endosperm is destroyed the more quickly because there is added to the handicap imposed upon it by a proliferating endothelium the demands of a relatively aggressive embryo. Being inherently incapable of attaining its normally dominant physiological position in the young seed, the hybrid endosperm becomes prey both to the maternal and embryonic tissues which impinge upon it.

A principal question to which an answer is sought in the present investigation—namely, whether the seed failure associated with matings between a diploid and its autotetraploid is comparable in kind to that occurring in an interspecific cross—is answerable in the affirmative. The histological evidence clearly shows that substitution of male gametes from either the autotetraploid (n=24) or *L. peruvianum* (n=12) for normal male gametes in fertilization of an ovule of diploid *L. pimpinellifolium* (n=12) precipitates a common chain of abnormal development events culminating in seed collapse. The seeds resulting from the 4n×2n mating show the same series of histological changes preceding breakdown as 2n×4n seeds. The number of chromosomes in the 4n×2n endosperm is 60 (fig. 20), whereas that in the 2n×4n endosperm would be 48.

In its genetic organization the angiosperm seed is a mosaic. Normally the cells in the maternal tissue and the embryo possess the same number of chromosomes although not necessarily the same genes. The endosperm, whose mother cell is a product of triple fusion, carries an additional chromosome set of maternal origin. In the simplest case, therefore, the endosperm is 3n, whereas embryo and the maternal tissues of the seed are 2n. The chromosome numbers in the three parts of the various classes of tomato seeds under investigation are given below. The symbols "n" and "n_1" refer to the haploid sets of *L. pimpinellifolium* and *L. peruvianum* chromosomes, respectively. The matings which fail to yield plump seeds are boxed.

The evidence is clear in the writers' judgment that the embryo, as an agent

contributing to the present type of seed failure, can usually be ignored. At most its rôle can be only secondary to that of the larger, more active endosperm. It is the latter tissue in its interrelationships with the maternal portion of the young seed that determines primarily whether development succeeds or fails. Persistence of the antipodals as in the juvenile caryopsis of the Gramineae complicates the relationship of the endosperm to the outer tissues of the seed but does not alter its basic significance. A primary requirement for development of the embryo is that the latter establish an efficient connection with a food supply which is mainly outside the seed. In the Gramineae, as in other families, it is the endosperm which mediates the nutritive arrangements (COOPER and BRINK 1944; BRINK and COOPER 1944).

NUMBER AND ORIGIN OF CHROMOSOMES

	(1)		(2)		(3)	
	2n×2n		2n×2n$_1$ (*peruv.*)		2n×4n	
Maternal tissue	24	nn	24	nn	24	nn
Endosperm	36	nn·n	36	nn·n$_1$	48	nn·nn
Embryo	24	n·n	24	n·n$_1$	36	n·nn

	(4)		(5)		(6)	
	4n×4n		4n×2n$_1$ (*peruv.*)		4n×2n	
Maternal tissue	48	nnnn	48	nnnn	48	nnnn
Endosperm	72	nnnn·nn	60	nnnn·n$_1$	60	nnnn·n
Embryo	48	nn·nn	36	nn·n$_1$	36	nn·n

The table above shows that the difference between the viable 2n×2n and the inviable 2n×4n classes of seeds (columns 1 and 3) lies in the latter possessing the tetraploid rather than the triploid number of chromosomes in the endosperm. The chromosome number of the associated maternal tissue in these two cases, of course, is the same. Similarly, the 4n×2n seed (column 6) with the pentaploid number of chromosomes in the endosperm fails, whereas the 4n×4n seed (column 4), differing in having a hexaploid endosperm, succeeds. The ratio of chromosome numbers between endosperm and maternal tissue in the viable 2n×2n and 4n×4n seeds is identical, namely 3:2. Increasing this ratio to 4:2 in the one case and decreasing it to 5:4 in the other, results in failure of the seeds.

There is at present no clue to the reason why these alterations in chromosome balance between endosperm and maternal tissue should radically disturb seed development. Data from numerous sources agree in showing that doubling the chromosome number increases nuclear size, and the change is often very close to proportional. Concomitant enlargement of the cell is usual. It would be difficult to determine in the young endosperm the effect on nuclear and cell size of variations in chromosome number because of the meristematic condition of the tissue and the hypertrophy that commonly occurs, not to mention

the variation in shape of these structures. So we have not attempted it. Casual observation, however, reveals that there is a general tendency in both endosperm and associated maternal tissue for cell and nuclear size to rise as the chromosome number increases. A basis for possible difference in physiological behavior lies in the fact that as nuclear and cell volume are altered, the surface area of these structures does not change proportionately. Were the bodies spherical, the surface area would vary as the two-thirds power of the volume. Assuming that surface-volume relations of nucleus and cell are important in certain metabolic processes, the relatively large differential occasioned by a doubling of volume might well affect growth significantly. A balanced change in chromosome number, as in the tetraploid, does not affect seed development adversely. The incompatibility arises when the chromosome ratio between maternal tissue and endosperm is varied in either direction. If surface-volume relations in cell and nucleus are involved in seed abortion, evidently it is the differential set up across the boundary between endosperm and endothelium which is important.

The mating $2n \times 2n_1$ (*L. peruvianum*) is an example of a cross which leads to seed collapse, although no alteration from the normal in chromosome balance between endosperm and maternal tissue is involved. The literature affords several other instances of the same kind. For convenience the cause of failure in these cases may be termed genic in contrast with the chromosomal type just considered.

Comparison of the $4n \times 2n$ mating in *L. pimpinellifolium* with the $4n \times 2n_1$ (*L. peruvianum*) mating shows that the genic and chromosomal factors affecting seed development may interact to give effects which are not predictable from a knowledge of the action of each factor alone. Seeds from the former mating collapse, whereas many from the latter develop. The essential difference between the two kinds of seeds resides in the genes carried in one of the five sets of chromosomes in the respective pentaploid endosperms (columns 5 and 6). The fact that $4n \times 2n$ seeds abort indicates that a 5:4 ratio in chromosome number between endosperm and maternal tissue is unfavorable. Likewise, collapse of the seeds resulting from the $2n \times 2n_1$ (*L. peruvianum*) cross shows that substitution of a *peruvianum* for a *pimpinellifolium* genome in the triploid endosperm associated with diploid maternal tissue is fatal to seed development. One might expect from these facts that the $4n \times 2n_1$ (*L. peruvianum*) mating would lead to abortive seeds, since it involves a seemingly unfavorable chromosomal balance and an unfavorable genic substitution as well. Such, however, is not the case. Many $4n \times 2n_1$ (*L. peruvianum*) seeds grow to maturity.

It would seem that continued development of the young seed requires the maintenance of a delicate physiological balance between endosperm and adjacent maternal tissue. This balance appears to be upset in one way by altering the chromosomal relations and in a different way by genome substitution. The ultimate effects of both kinds of primary disturbance, however, are alike, since both precipitate the same series of histological changes leading eventually to

seed failure. It must be supposed that the two types of unbalance are opposite in direction to some extent so that when combined, as in the 4n×2n$_1$ (*L. peruvianum*) seed, they tend to cancel each other. These considerations illustrate the difficulty involved in assigning the cause of seed failure when the latter arises in crosses between species which differ both in chromosome number and genic complement.

SUMMARY

A comparative histological study of seed development following reciprocal matings between diploid and tetraploid strains of *Lycopersicon pimpinellifolium* and crosses of these races with *L. peruvianum* is reported.

A high percentage of the flowers pollinated formed mature fruits in all matings except 2n×4n. The flowers and young fruits in the latter case were shed within eight days. The initial set was high in the 4n×4n mating, but numerous immature fruits were shed at intervals up to 24 days.

Practically all the seeds formed following the 2n×2n mating were plump at maturity. About two-thirds of those found in the 4n×4n fruits were sound and the remainder shrivelled. The 4n×*L. peruvianum* cross likewise gave a relatively high proportion of plump, although small, seeds. The seeds in the mature fruits resulting from the 4n×2n and 2n×*L. peruvianum* matings, on the other hand, were much shrunken and incapable of germination.

Double fertilization takes place and both endosperm and embryo start growth in all developing seeds. Fertilization was found to occur approximately 24 hours following pollination of 2n flowers but is somewhat delayed in the 4n flowers regardless of the type of mating.

The endosperm develops rapidly in the 2n×2n and 4n×4n seeds and differentiates early, the peripheral layer of cells acting as an absorbing tissue. The endothelium—that is, the inner layer of cells of the expanding integument adjacent to the endosperm—remains a single layer of densely cytoplasmic cells in the growing seed. Several layers of cells of the integument immediately outside the endothelium become empty of their contents during the early stages of development of the endosperm, thus serving as a source of nutrients.

The chalazal aperture opposite the end of the vascular bundle in conjunction with the endothelium serves as a pathway for nutrients at later stages of development of the endosperm.

The embryo grows at the expense of the endosperm.

The development of the 4n×*L. peruvianum* seed is essentially similar although much slower than that of the 4n×4n seed.

Growth of the endosperm in the 2n×4n, 2n×*L. peruvianum*, and 4n×2n seeds is slow, and the cells at the chalazal end are large and highly vacuolate at 144 hours. By this time there is a conspicuous thickening of the endothelium mainly on the dorsal side of the endosperm. Shortly thereafter the endothelium becomes actively meristematic and grows rapidly so that this tissue has within a few days completely filled the space formerly occupied by the endosperm.

A large amount of deeply-staining material accumulates in the cells of the chalazal pocket during the course of collapse of the aborting endosperms.

The embryo grows slowly and is very small and starved in appearance by the time it has become completely surrounded by the hyperplastic endothelium. Shortly thereafter the embryo dies, and a much-shrivelled seed is found in the mature fruit.

The course of seed failure associated with matings between the diploid and its autotetraploid is very similar to that found following the interspecific cross.

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