A STUDY OF VIVIPAROUS MUTANTS OF MAIZE

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ABSTRACT

The meaning and extent of vivipary among the higher plants are considered. The nature of vivipary in maize as reported by earlier workers is compared with observations made in this study. The use of interchanges among chromosomes of the basic set (A-type chromosomes) and of interchanges between A and B-type chromosomes in genetic studies is outlined. The results of genetic studies with six viviparous mutants are reported. Experiments involving the use of embryo culture techniques to elucidate the cause of vivipary in maize are described. These experiments revealed that viviparous$_5$ embryos are more tolerant of high carbon dioxide concentrations than normal embryos. Experiments to check the role of the cob in preventing premature germination, as well as experiments to determine if acetaldehyde plays a similar role, yielded negative results. The total auxin contents of viviparous and normal seeds are reported. No obvious differences between the two classes of seeds with regard to this growth substance was found. The significance of the genetic and embryo culture studies as it relates to vivipary in maize is considered.

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INTRODUCTION

Most plants are capable of surviving periods unfavorable for their growth. In general this is done by producing specialized structures such as seeds, spores or zygotes which are capable of withstanding more extreme conditions than the vegetative body of the plant.

Among the algae the zygote resulting from the fusion of two gametes frequently secretes a thick wall about itself and remains in a resting state until conditions are favorable for vegetative growth. In the fungi, zygotes and spores are employed to bridge periods of adverse growing conditions. Members of the Bryophytes and Pteridophytes rely on spores. The spores and zygotes produced by these lower plants are relatively undifferentiated, frequently consisting of a single cell which upon germination produces the vegetative structures.

In the higher plants the seed is utilized for this purpose. Unlike spores or zygotes a seed is a complicated structure, being composed of many cells and shows a high degree of differentiation. Within it is found the embryo which upon germination yields another plant like its parents. The seed is designed to fulfill the needs of this embryo. There are usually found outer coats which serve to protect the embryo from the external environment, and internal storage tissues which supply the required nutrients while the embryo is germinating.

Such a complicated structure undoubtedly involves many complex reactions, most of which are essential to the development of the seed and if interfered with by a mutation of one of the controlling genes will result in aborted seeds. However, reactions which can be disrupted without producing lethal changes are to be expected. Changes which are responsible for waxy, sugary, shrunken and brittle endosperm types in maize are examples of such non-lethal effects.

After the egg nucleus is fertilized, the resulting zygote starts its embryonic development. Such development produces not just a diminutive copy of the parental plant, but rather a structure specialized for its role in the propagation of the species.

It has been determined experimentally that maize embryos are capable of germination soon after pollination (La Rue, 1936; Haagen-Smit et al. 1945), yet in the developing caryopsis this does not take place. This suggests the presence of a mechanism for preventing precocious germination since the young embryo has an excellent food source in the endosperm, together with an adequate water supply. Some suggestions as to what this controlling mechanism is will be made in a later section.

At maturity the mechanism for inhibiting germination is lost but the seed is incapable of germination due to its desiccated condition. However, if this controlling mechanism is lost prematurely, before the seed is

too dry to permit germination, premature germination of seeds will result. Such seeds are occasionally observed and in some lines of maize the characteristic appears to be under genic control. These premature germinating seeds will be called viviparous, the term used by Eyster (1931b) to describe these mutants in maize.

Ascherson (1875) described apples which contained premature germinating seeds. In 1880, Treichel reported observing the germination of wheat and rye on the stalk. McKellar (1898) observed germinating seeds within a melon. He also observed seedlings in lemons, oranges, cucumbers, pumpkins, papaws and other fleshy fruits. Oppenheimer (1922b) indicates that Savelli (1920) had observed seeds germinating in the capsule of Nicotiana rustica, Citrus limonum and seedlings on ears of corn. Bonner (unpublished) obtained a variety of tomatoes which had a tendency to produce germinating seeds within the fruit.

One of the earliest references to vivipary in maize is that made by Savelli (1920). Mangelsdorf (1923) and Lindstrom (1923) briefly describe ears segregating viviparous seeds. Eyster (1924a) described a viviparous mutant which was genotypically similar to those described by Mangelsdorf and Lindstrom. Also in this paper, he considers briefly the significance of the association of white endosperm color and albino seedlings. In a second paper the same year Eyster (1924b) described a second viviparous mutant phenotypically identical to the

first one.

Mangelsdorf (1923) termed his mutants "germinating seed" (ge) while Eyster (1924) named his "primitive sporophyte", a term he chose because, "the sporophytes of all non-seed-bearing plants, which presumably are more primitive than seed-bearing plants are normally viviparous" (Eyster, 1931b). In his later paper Eyster (1931b) used the term "vivipary" (vp) to describe these mutants.

The papers mentioned above were solely descriptive in nature and aside from the allelic test reported by Eyster (1924b) in his second paper, no genetic studies were reported.

Mangelsdorf (1926) described five different viviparous mutants, including the mutant of his previous paper and the one described by Lindstrom (1923) the same year. He also postulated four additional genes to account for some ratios encountered in segregating ears. In addition he reported an indication of linkage between ge_1 and the gene for sugary endosperm in the fourth chromosome. Considered also in this paper is the relationship of vivipary to the lack of chlorophyll in the seedlings and to the endosperm color of the seed.

In a later paper, Mangelsdorf (1930) described the results of intercrossing the various viviparous mutants considered in his previous papers. Ears were obtained which segregated viviparous seeds in atypical ratios. These are explained by assuming five additional genes.

Also described in this paper is a viviparous mutant which has pink endosperm color as well as pink plumules. This mutant is probably the same as one described by Sprague (1936) which is allelic to $\underline{vp_7}$ of this paper. Mangelsdorf (1930) also indicated the possibility of an additional gene which had not been tested against his other stocks. In all Mangelsdorf demonstrated six different loci responsible for premature germination and postulated an additional nine loci to explain aberrant ratios. Eyster (1931b) questioned the advisability of calling into service additional genes to explain spurious ratios when dealing with a character such as vivipary which can be readily influenced by environmental conditions. In his paper linkage relations of four viviparous mutants are reported. These reported linkages will be considered in detail later.

THE NATURE OF VIVIPARY IN MAIZE

On the basis of endosperm and seedling phenotype which accompany the viviparous condition, the various viviparous mutants of corn can be divided into three classes, as follows: (1) those with yellow endosperm which produce green seedlings, (2) those with white or pale yellow endosperm which produce albino seedlings if grown in the light, but which have a bluish-green tinge if grown in the dark, and (3) those with yellow-

pink endosperm which produce seedlings that lack chlorophyll but have a faint pink color when grown in the light, but in addition will have a bluish-green tinge if grown in the dark.

These three classes of viviparous seeds have previously been reported by various workers. Mutants of class one have been reported by Mangelsdorf (1926, 1930) and Eyster (1931b). Mangelsdorf (1923, 1926, 1930), Lindstrom (1923), and Eyster (1924a, b, 1931b) describe mutants of class two and brief descriptions of class three mutants are given by Mangelsdorf (1930) and Sprague (1936).

A. Viviparous Mutants of Class One

This group of mutants is represented by $\underline{vp_1}$ and $\underline{vp_4}$ (which appear to be allelic) and $\underline{vp_8}$. These mutant genes do not visibly influence carotinoid or chlorophyll development. $Viviparous_1$, however, differs from other viviparous types in that seeds homozygous for this gene show little or no development of aleurone color. (The genetical evidence for this will be considered in the section on genetics). Vivipary in this mutant does not commence until late in the ontogeny of the caryopsis at about the time aleurone color is developing. This suggests the possibility that germination might interfere with development of color in the aleurone layer. However, aleurone color does develop in $\underline{vp_2}$, $\underline{vp_7}$, and $\underline{vp_9}$, kernels,

all of which germinate at an earlier period in ontogeny than $\underline{vp}_1$. This suggests that germination *per se* is not responsible for this color inhibition but must be the result of some peculiar action of the $\underline{vp}_1$ gene.

It is not known if $\underline{vp}_8$ affects aleurone color. This mutant is phenotypically and genetically distinct from $\underline{vp}_1$.

Premature germination of $\underline{vp}_8$ kernels is not too reliable. On some ears only a small percentage of the seeds are viviparous. However, there is always present another class of seeds characterized by being very shrunken in the general region of the germ as if the endosperm had been digested in this area. If these seeds are combined with the viviparous class, the expected 3:1 ratio is very closely approximated. In spite of poor germination it is possible to obtain mature homozygous $\underline{vp}_8$ plants. The viviparous seeds produce weak, dwarf seedlings which have short internodes and short pointed leaves. The mature plants are very dwarfed and often produce leaves extending from the plant in all directions. Such a plant is pictured in Figure 1. Most of these plants produce functional tassels, but only a few are strong enough to produce an ear. When F_1 plants of a cross of $\underline{vp}_8$ and standard are self-pollinated, both viviparous seeds and seeds with shrunken germs are produced. The two classes of abnormal phenotypes total approximately $\frac{1}{4}$ of the seeds. The conditions responsible for the production

FIGURE 1

Homozygous Viviparous$_8$ Plants

1a. 1b.

Fig. 1a. Dwarfed viviparous$_8$ plant illustrating the leaf arrangement that frequently occurs.

Fig. 1b. Two homozygous viviparous$_8$ plants showing the more typical dwarfed condition.

of these classes of seeds are not understood. A histological study of this mutant during the development of the caryopsis would be helpful in answering this problem. Because these seeds cannot be distinguished until late in ontogeny, such a study has not been undertaken. By utilizing an appropriate technique, it should be possible to select the $\underline{vp}_8$ seeds by the early milk stage. This technique involves the use of a translocation between chromosomes 1 and 6 which is closely linked to $\underline{vp}_8$ and the gene for yellow endosperm (Y_1). If a plant heterozygous for such a translocation, $\underline{vp}_8$ and Y_1 is self-pollinated or backcrossed to a white-endosperm-viviparous$_8$ plant, endosperm color will be closely linked to $\underline{vp}_8$, and thus it will be possible to distinguish between $\underline{vp}_8$ and normal seeds at an early stage in development. This technique will be discussed in more detail in the section on linkage methods.

B. Viviparous Mutants of Class Two

Viviparous$_2$, viviparous$_5$, and viviparous$_9$ are representative of this class of mutants, which are interesting because of the apparent lack of carotinoids and chlorophyll development accompanying the expression of vivipary.

Bandurski (unpublished) measured the total carotinoid content of seedlings of viviparous$_5$ on a Beckman Model D. U. Spectrophotometer at 440 mμ. He found the total density at this wave length was less than 5% of the density

of a similar weight of normal material. No suggestion of any compound possessing typical carotinoid absorption was observed.

The relationship of vivipary to the inhibition of carotinoids and chlorophyll development was interpreted differently by Eyster (1924a, b, 1931b) and Mangelsdorf (1926). Eyster assumed close linkage between the gene responsible for vivipary and another gene which inhibits chlorophyll and carotinoid formation. He reported obtaining twenty-two pale non-viviparous seeds which yielded twenty-one green seedlings and one albino seedling upon germination. These twenty-two seeds he considered to be crossovers (Eyster, 1924a). These results can be explained by hetero-fertilization and will be considered below. In 1931 he reported obtaining a few green viviparous seedlings which, when grown to maturity, produced a chlorophyll-bearing strain of viviparous maize. He did not report any tests to determine if the derived chlorophyll-bearing strain was allelic to the original albino mutant. Since this was not done the possibility that there was a genetically distinct chlorophyll-bearing strain segregating in his stock has not been excluded.

Mangelsdorf (1926) did not agree with Eyster's interpretation of close linkage. He pointed out that it was extremely unlikely that so many viviparous mutants would occur, each closely linked to a factor responsible for producing white endosperm and albino seedlings. At

least nine such mutants from different sources have been described.

Mangelsdorf (1926) also observed that some of the white seeds on segregating ears were dormant, and as was the case with those found by Eyster in 1924, these produced green seedlings. But, whereas Eyster reported only on the seedling stage, Mangelsdorf grew his plants to maturity and self-pollinated them. If this type of seed is the result of crossing over, the self-pollinated ear should produce only white seeds. This was not the case. All of these ears segregated yellow and white seeds. In this study plants from nine such seeds have been grown and self-pollinated. All the resulting ears were found to be segregating for white endosperm and albino viviparous seedlings.

Mangelsdorf (1926) explained such exceptional seeds as a physiological correlation between vivipary, endosperm color and chlorophyll formation. He sectioned immature viviparous seeds and reported that there was evidence of germination even at the early milk stage, which suggested to him the possibility that the process of germination interfered with color deposition. On this interpretation he explained the two distinct classes of viviparous mutants. Those which start germinating at an early stage interfere with color deposition and, therefore, result in white seeds, while those mutants that are characterized by yellow endosperm do not start to germinate

until after color deposition has been accomplished. The exceptional classes are explained as the result of variations in this mechanism.

This explanation is inadequate for the following reasons:

1. Mutants used in this study have shown no obvious signs of germination (i.e., elongation of the coleoptile and root or enlargement of the scutellum) until late in the development of the seed. Germination starts in $\underline{vp_5}$ seeds about 26 to 29 days after pollination while seeds of $\underline{vp_9}$ do not show signs of germination until 32-39 days after pollination. This is considerably later than the initiation of color in the endosperm.

2. Occasionally ears segregating $\underline{vp_5}$ have been found with a high frequency of white seeds that become dormant. Since these seeds did not germinate, it would be expected, under Mangelsdorf's interpretation, that they should be yellow.

3. If the exceptional classes of seeds are explained by chance variation of the germinating mechanism, it would be expected that these chance variations occurring in seeds of viviparous mutants characterized by yellow endosperms and green seedlings would occasionally produce a seed with a white endosperm. These are never found.

Sprague (1936) explained the existence of analogous classes of seeds on ears segregating for $\underline{vp_7}$,

as the result of hetero-fertilization. This term was first used by Sprague (1929) to describe exceptional seeds which have embryos and endosperms of different genetic constitutions. In a later paper such seeds were shown to result from the fusion of the egg and polar nuclei with sperm nuclei from two pollen grains of unlike genotypes (Sprague, 1932).

Upon self-pollinating a plant heterozygous for one of the albino-pale-endosperm-viviparous mutants occasional seeds that are the product of hetero-fertilization are expected. Only seeds resulting from female gametophytes in which the egg nucleus and polar fusion nucleus both carry the viviparous gene can show hetero-fertilization. If the egg nucleus is fertilized by a sperm from a pollen grain carrying a non-mutant allele and the polar fusion nucleus unites with a sperm from a pollen grain which carries the mutant allele, the resulting seed will have a pale endosperm and a non-viviparous embryo. The twenty-one non-viviparous-white endosperm seeds which produced green seedlings reported by Eyster (1924a) and similar ones reported by Mangelsdorf (1926) can be explained as the product of such hetero-fertilization. All plants resulting from such seeds which have been grown to maturity and self-pollinated have produced ears which segregate for vivipary. Mangelsdorf (1926) realized that this class of seeds was difficult to explain by his hypothesis since the embryos remain dormant and

therefore color deposition should proceed unhindered by starch digestion induced by a germinating embryo. This class of seed is readily explainable on the basis of hetero-fertilization.

The yellow seeds which produce albino viviparous seedlings described by Mangelsdorf (1926) are recognized as the product of the reverse combination in which the polar nuclei unite with the sperm from a pollen grain carrying the normal allele while the egg nucleus is fertilized by the sperm from the pollen grain carrying the mutant allele.

Both classes of exceptional seeds described by Mangelsdorf (1926) and Eyster (1924a, b, 1931b) are readily explainable as the result of hetero-fertilization.

Mangelsdorf (1926) suggested that the absence of chlorophyll in the viviparous seedlings of this class of mutants might result from the inhibition of chlorophyll formation by the act of premature germination of the viviparous embryos. In support of this hypothesis he describes some viviparous seeds which occasionally are found to have chlorophyll. According to his hypothesis these are green because the premature germination started too late to interfere with chlorophyll formation. He also pointed out that he had never found a dormant white seed which produced an albino seedling.

Mangelsdorf's hypothesis and the two pieces of corroborative evidence are inadequate for the

following reasons:

1. Fyster (1924a) reports finding one white dormant seed out of twenty-two which produced a white seedling on germinating. This type of seed is quite frequent in some of the stocks of viviparous$_5$.

2. The fact that an occasional chlorophyll-bearing viviparous seed is obtained is not in itself corroborative evidence for his hypothesis since ears that are homozygous for the normal alleles of vivipary occasionally yield one or more viviparous seeds as the result of environmental factors. Until the plants from this class of seeds are grown, self-pollinated and backcrossed to the original viviparous stock to determine if they are homozygous for vivipary and, if so, if they are the same viviparous mutants as the stock from which they arose, such a seed can be considered as one originating from environmental stimulation.

3. The most convincing evidence against Mangelsdorf's hypothesis is the result of studies involving the culturing of excised corn embryos. Eleven day old embryos were removed from seeds which were too immature to have produced endosperm color. These were transfered to a culture medium enriched by a water extract of mature corn. Embryos placed on such a medium cease embryonic growth and proceed to germinate. Most of these embryos were too immature to produce normal growth, but

several did succeed in producing a leaf or two which were normal with respect to chlorophyll formation. If Mangelsdorf's hypothesis is correct these seedlings would be expected to lack chlorophyll because they germinated quite prematurely.

The preceding three points make it very unlikely that the albinism associated with vivipary is the result of the premature germination of the embryo.

If the so-called crossover classes mentioned by Eyster (1924a, b, 1931b) and exceptional seeds reported by Mangelsdorf (1926) are the result of hetero-fertilization, it would appear that the one mutant gene expresses itself in three ways phenotypically (i.e., inhibits the formation of carotinoids in the endosperm and seedling, inhibits chlorophyll formation and induces premature germination). Although the step affected by this gene is not known, it is possible that some step leading to the production of carotinoids is blocked through its action. This will account for the lack of carotinoids in both the endosperm and embryo of the mutant seeds. It is interesting to note that there are no reports of plants with chlorophyll without carotinoids also being present. This has led some workers to suggest that the phytyl side chain of the chlorophyll molecule might be derived from a carotinoid or some closely related substance. If this is the case, then it is obvious that a gene inter-

fering with the production of carotinoids could also inhibit the formation of chlorophyll.

Tatum (1949) and Davis (1950) have reported mutant strains of Neurospora crassa and E. coli, respectively, which have multiple requirements for growth substances. These writers postulate a gene block to the formation of a common precursor of all the required growth substances. This suggests a mechanism by which a single gene could produce multiple phenotypic effects as those observed in viviparous strains of corn.

C. Viviparous Mutants of Class Three

Viviparous$_7$ is the only representative of this class. The pink color produced by this mutant can be observed in the developing caryopsis at the time yellowing of the endosperms of normal seeds on the same ear takes place. The immature endosperm is an intense pink which fades as the seed matures, becoming more of an orange color apparently due to the laying down of yellow pigments. At maturity these seeds are sometimes rather difficult to separate, by color of the endosperm alone, because the pink pigment becomes masked by the yellow.

Preliminary studies of the carotinoid content of mature seeds of this mutant revealed that it is strikingly different from that found in normal seeds segregating on the same ear. Separation of the various carotinoid components from a petroleum ether extract of vp$_7$

seeds by absorbing them on a magnesium oxide chromatographic column revealed that these seeds lacked most, if not all, the color bands found with a similar analysis of normal seeds. In addition, the viviparous seeds showed several carotinoid-like bands that did not appear in the normals. These various components of normal and viviparous seeds have not been identified. However, the preliminary results indicate that the $\underline{vp}_7$ gene alters, very markedly, the normal carotinoid development of the seed.

Additional evidence that this gene is involved in the carotinoid metabolism of the plant is demonstrated by combining it with the white allele of the gene for yellow endosperm found on chromosome six. Seeds that are homozygous for the white allele and also for $\underline{vp}_7$ have white endosperms and pink viviparous embryos. White seeds not carrying the $\underline{vp}_7$ allele have endosperms lacking in carotinoid pigments, but produce seedlings with normal carotinoid and chlorophyll development. In the double recessive seeds no pink color develops in the endosperm, but the viviparous$_7$ embryo is pink. Thus, in the endosperm where no carotinoid pigments are produced no pink pigment is formed, and in the endosperm where normally carotinoids are produced the pink pigment is found.

The lack of chlorophyll in this mutant again emphasizes the dependence of this pigment on carotinoid production.

D. A Summary of the Characteristics of the Viviparous Mutants

The characteristics of all viviparous mutants considered in this study are summarized in Table 1.

TABLE 1

	Time of germ.	Color of aleurone in presence of A_1A_2CRPr	Color of endosperm	Color of seedling in light	Color of seedling in dark
vp_1	late dough	colorless	yellow	green	-
vp_2	late dough	purple	white	white	-
vp_5	middle dough	-	white	white	bluish-green tinge
vp_7	middle dough	purple	pink	pink	pink + bluish-green tinge
vp_8	exact time difficult to determine	-	yellow	green (dwarf plants)	-
vp_9	middle dough	purple	white	white	bluish-green tinge

SPECIAL TOOLS USED TO LOCATE GENES

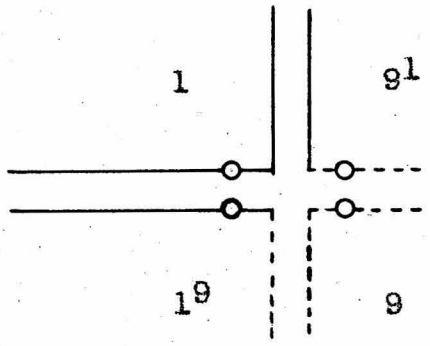
Besides the conventional method of locating genes by obtaining indications of linkage with known genes, two special tools were used; namely, translocations between different chromosomes of the basic set and trans-

locations involving a chromosome of the basic set and a B-type chromosome. An outline of the use of these tools for locating unknown mutants will show the advantage gained by employing them.

A. Translocations

In maize, mutant genes affecting the endosperm are some of the best genes for linkage studies, but the number of good endosperm mutants which can be used for this purpose is limited. This handicap can be overcome by choosing the proper translocations. For example, if a translocation is selected which involves chromosome nine in the region of the waxy gene and has the other break in chromosome one it is possible then to show linkage between waxy and genes in the first chromosome.

Translocation $1\text{-}9_a$ will be used to demonstrate this technique. We can represent the configuration formed by these chromosomes in a plant heterozygous for the translocation diagrammatically as follows:



The unaltered chromosomes are indicated by the numbers 1 and 9, while 1^9 represents the translocated chromosome which has the centromere of 1 with the translocated piece of 9 attached and 9^1 represents the translocated chromosome which has the 9 centromere and the translocated piece of chromosome 1 attached.

The several different 2-2 combinations which result from the segregation of these chromosomes at meiosis are as follows:

$1 - 9$
$1^9 - 9^1$ resulting from alternate segregation,

$9^1 - 1$
$1^9 - 9$ resulting from adjacent-one segregation,

$1 - 1^9$
$9 - 9^1$ resulting from adjacent-two segregation.

According to Burnham (1949, 1950) adjacent-two segregation occurs only when no crossing over takes place in the interstitial segment.

Microspores produced following alternate segregation have balanced chromosome complements, either 1, 9 or 1^9, 9^1. These develop into normal looking functional pollen grains well filled with starch. The corresponding microspores produced following adjacent segregation have unbalanced chromosome complements and develop into non-functional abortive pollen grains which may be empty or partly-filled with starch. Alternate and adjacent segre-

gations occur with equal frequency giving rise to pollen which is "semisterile". Semisterility produced by such a heterozygous translocation behaves like a dominant character and can be used as such. Since the only functional gametes produced by a plant heterozygous for the translocation are those carrying chromosomes one and nine and those carrying 1^{9} plus 9^{1}, the point of translocation in the two translocated chromosomes must always be included in the same gamete and thus these points are completely linked. Similarly the corresponding points on the untranslocated chromosomes must be completely linked. Therefore, genes in one chromosome closely linked to the point of breakage will be linked to genes in the other chromosome close to the translocation point.

By selecting the appropriate translocations it is now possible to use the few good endosperm genes to test for linkage in all chromosomes of the complement. Stocks of translocations which have one break in chromosome nine close to the waxy (wx) locus while the other breaks are in various of the other chromosomes and similar stocks of translocations involving the recessive gene sugary (su) have been established. Such translocations enable one to use the excellent genes, wx and su, for linkage studies in all chromosomes of the complement.

To locate an unknown gene that is not linked to wx or su crosses can be made to stocks of translocations closely linked to these genes. The F_2 ratios from

TABLE 2

Translocations Used in This Study
Break positions as listed by various workers.

Translocation	Burnham (1934)	Anderson (1938)	Anderson (1939)	Anderson & Brink (1940)	Anderson (1941)	Longley (unpubl.)
1-4a						1 L.49
						4 S.66
2-4a						2 L.29
						4 L.15
2-4c						2 L.77
						4 S.09
$2\text{-}4_{K10}$						2 S.19
						4 L.30
4-5d						4 S.21
						5 L.19
4-6a						4 L.33
						6 L.44
4-8a			4 S.5			4 S.54
			8 L.1			8 L.48
4-9a		4 L.1				4 L.18
		9 L.8				9 L.50
$4\text{-}10_{B45}$						4 S.70
						10 L.11
1-9a						1 S.17
						9 L.17
1-9b		1 L.6				1 L.42
		9 L.5				9 L.54
2-9b		2 S.1				2 S.12
		9 L.2				9 L.12
3-9a		3 -				3 L.19
		9 L.1				9 L.40
3-9c		3 L.1		3 L.1		3 L.15
		9 L.2		9 L.2		9 S.20
4-9b		4 L.6				
		9 L.2				
5-9a	5 L.7					5 L.80
	9 S.0					9 S.21
6-9a						6 S.79
						9 L.40
$6\text{-}9_{X\ 25\text{-}78}$						6 L.17
						9 L.22
$7\text{-}9_{a\ 76}$						7 L.27
						9 L.20
9-10b		9 L.3				9 S.11
		10 near s.a.				10 S.28
1-7c						1 L.34
						7 L.14
1-3a				1 S.2	1 S.25	1 S.19
				3 L.2	3 L.2	3 L.15

such crosses can be observed for any indication of linkage with wx or su. Indications of linkage with these genes will then reveal that the unplaced gene is on the other chromosome involved in the translocation. The translocation can also be used as a marker in a backcross test to determine the position of the gene on the chromosome.

The break positions of the translocation can be established cytologically and thus give some indications as to the physical location of the gene on the chromosome.

Use has been made of translocations in this study to locate a chromosome carrying an unknown gene and also in mapping positions of genes.

The translocations used in this study and the cytological positions of the breaks are given in Table 2.

B. Translocations Involving B-Type Chromosomes

Besides the basic set of chromosomes (A-type) maize may also contain various numbers of supernumerary chromosomes which have been designated as B-type. In a given population the number of B-type chromosomes may vary from plant to plant. Randolph (1941) indicates that plants with from one to ten of these supernumerary chromosomes are phenotypically indistinguishable from those lacking them.

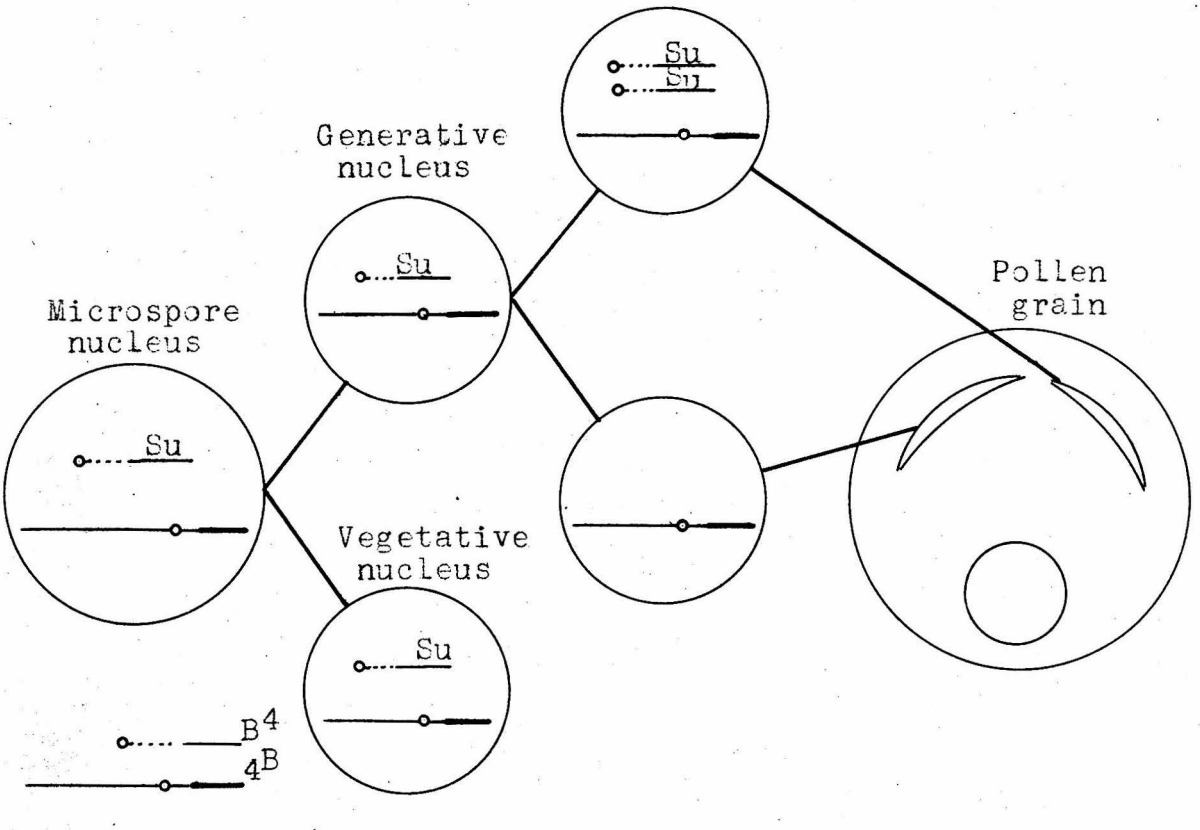
Roman (1947, 1948) has described the behavior of two A-B interchanges. He was able to show by using

A-B interchanges that the unusual inheritance pattern of the B-type chromosomes, as reported by Randolph (1941) and Longley (1927), was the result of non-disjunction in the male gametophyte of the B centromere at the division of the generative nucleus to produce the two gametic nuclei.

The behavior of the translocation between a B-type chromosome and the short arm of chromosome four in the development of the pollen grain is diagrammatically shown in Figure 2.

As the result of non-disjunction of the B centromere the two gametic nuclei differ in respect to their genetic constitution. One has the translocated portion of chromosome four in duplicate (hyperploid gamete), while the other is deficient for this section (deficient gamete). Both of these gametes are functional, thus, there are two possibilities when fertilization takes place: (1) the hyperploid gamete can fertilize the egg and the deficient fuse with the polar nuclei, and (2) the hyperploid gamete can fuse with polar nuclei and the deficient gamete fertilize the egg. It is, therefore, possible by using A-B interchange plants as pollen parents to obtain seeds with embryos or endosperms that are deficient for a section of a chromosome. For this reason A-B interchanges can be used to locate unknown recessive genes. The recessive character will be observed in the F_1 progeny if the gene

FIGURE 2



The probable behavior of translocation B-4a in the development of the pollen grain (Redrawn from Roman, 1947, p. 399).

is located in the portion of the chromosome that is attached to the B centromere (Roman 1947).

A-B interchanges are also useful in studying embryo-endosperm interactions of some of the viviparous mutants. If the gene responsible for vivipary is located in a section of a chromosome that has been translocated to a B centromere, then two classes of seeds are readily obtainable by pollinating a plant carrying the mutant allele with this A-B interchange pollen. In such a cross, if the deficient gamete fuses with the egg, and the hyperploid gamete fuses with the polar nuclei, a mutant-embryo, normal-endosperm seed is produced. Reciprocally, if the hyperploid gamete unites with the egg and the deficient gamete fuses with the polar nuclei, a normal-embryo, mutant-endosperm seed results.

The results obtained from employing A-B interchanges as outlined in the previous paragraph will be considered in a later section.

The A-B translocations used in these studies are listed in Table 3.

TABLE 3

A-B Interchanges Used in This Study

Interchange*	Cytological position of break points**	Position of break-points relative to marker genes
B-1a	L .2-.3	Proximal to hm
B-1b	S next to centromere	
B-4a	S .25	Proximal to su
B-7b	L .3	Between v_5 & ra
B-9a	L .5	
B-9b	S .4	Between wx and c; less than 1% c.o. between breakage point and wx
B-10a	L .3-.4	Proximal to g

* Number refers to the chromosome involved in the interchange.

** The position of the break point is given as the distance from the centromere in the long (L) or short (S) arm of the chromosome as a fraction of the total length of the arm.

GENETIC STUDIES INVOLVING VIVIPAROUS$_1$

Eyster (1924a) originally described viviparous$_1$ as a pale endosperm albino seedling mutant. In 1931 he reports obtaining a strain of green viviparous maize from the original stock of $\underline{vp}_1$. Such green viviparous plants have never been observed in stocks of $\underline{vp}_2$, $\underline{vp}_5$, and $\underline{vp}_9$ in the present author's cultures. These mutants are all characterized by pale endosperm and albino seedlings. Eyster reports no crosses between the green and albino strains of $\underline{vp}_1$ to determine if the two are allelic. Thus, there is a possibility that the original $\underline{vp}_1$ mutant and the derived green one might be different mutants.

The stock of $\underline{vp}_1$ obtained from the Maize Genetics Coöperation for this study was evidently derived from the green $\underline{vp}_1$ strain and this term will henceforth be used to indicate the $\underline{vp}_1$ mutant obtained from the Maize Genetics Coöperation. This mutant gene has no influence on endosperm color, except that it produces a pale crown on some of the more advanced viviparous seeds, and does not interfere with the production of chlorophyll.

Stocks of $\underline{vp}_1$ and $\underline{vp}_4$, also described by Eyster (1931b), obtained from the Maize Genetics Coöperation proved to be allelic when crossed together. A possible bookkeeping error during the time these mutants were being perpetuated could have been responsible for the two stocks carrying the same gene. However, Eyster (1931b)

does not report an allelic test between the two strains and his evidence for two different mutants seems to be based on different apparent linkage relations.

Viviparous$_1$ was reported as being linked to R with only 0.15% crossing over. As will be shown later there is a distinct possibility that this is only apparent linkage caused by inhibition of aleurone color when the vp$_1$ gene is homozygous. Using data derived from two F_2 ears totaling 496 seeds, Eyster (1931b) estimated that there was approximately 35% crossing over between viviparous$_1$ and the golden locus. The above evidence is not sufficient to place vp$_1$ on chromosome ten.

The evidence given for the location of vp$_4$ in chromosome nine is based on one F_2 ear heterozygous for this mutant and for shrunken (sh). This ear yielded the following classes of seeds: Sh Vp 200, sh Vp 14, Sh vp 22, and sh vp 87. Using these data he calculated 11.8 crossover units between vp$_4$ and sh (Eyster 1931b). That this is a poor ear to base F_2 linkage on can readily be seen by summing the non-viviparous and viviparous classes which give values of 214 and 109, respectively. Similarly, summing the non-shrunken and shrunken classes yields values of 222 and 101. These values deviate considerably from the expected 3:1 ratio.

The linkage data reported by Eyster (1931b) are not sufficient to eliminate possible allelism of the original viviparous$_1$ and viviparous$_4$ mutants. Hereafter,

$\underline{vp_1}$ will be used to designate progeny from the original $\underline{vp_1}$ and $\underline{vp_4}$ stocks.

If Hyster's linkage information is correct, this mutant should be either on the short arm of chromosome nine or the long arm of chromosome ten.

Crosses of B-10, which includes the loci of R and g, to three homozygous and seven known heterozygous $\underline{vp_1}$ plants proved conclusively that the mutant is not in the long arm of chromosome ten included in this translocation. Eight crosses of B-9b, which includes the loci of sh and wx, and seven crosses of B-9a, which includes the distal half of the long arm of chromosome nine, with plants known to be heterozygous for $\underline{vp_1}$, gave no indications that the mutant was in either of the sections of chromosome nine carried by these B centromeres.

Four F_2 ears heterozygous for $\underline{vp_1}$ and sh gave the results in Table 4. Both classes of viviparous seeds have been grouped together because of the difficulty in distinguishing the double recessive. This ratio closely fits the expected 9:3:4 ratio, having a chi square value of 1.3862. A deviation as large or larger than this is expected by chance about 50% of the time. This indicates that there is no linkage between this mutant and shrunken.

TABLE 4

F_2 Ratios from Plants Heterozygous for Shrunken and Viviparous$_1$

Plant	Vp Sh	Vp sh	vp
49-649-1	174	59	71
49-649-7	190	49	79
49-649-10	234	82	91
49-651-5	143	41	68
	741	231	309

The results of backcrosses of plants heterozygous for wx and vp$_1$ to wx vp$_1$ plants is given in Table 5. The calculated percentages of crossing over are 47.6 and 51.0, indicating no linkage between these two genes.

TABLE 5

Backcross Progenies of $\frac{+\ vp1}{wx\ +}$ and $\frac{wx\ vp_1}{wx\ vp_1}$

Parent family	Parental combinations		Recombinations		Total	Percent recombinations
	+ vp	wx +	+ +	wx vp		
49-5843	147	164	142	140	593	47.6
50-1607	1323	1274	1419	1386	5502	51.0

Crosses of viviparous$_1$ with B-9a, B-9b, B-10 and with the genes wx and sh show that vp$_1$ is not in chromosome nine nor in the distal portion of the long arm of ten.

Tests with B-1a, -1b, -4a, and -7b besides those previously reported have failed to reveal any further information as to the locus of this gene. As yet no indication of its position has been obtained but further crosses are being planned.

This mutant is of special interest because it inhibits the production of aleurone color in the presence of dominant A_1A_2C and R.

Table 6 gives the results of a backcross involving parents of the following genotype:

$$A_1A_1A_2A_2CcRrVp_1vp_1 \times A_1A_1A_2A_2CCrrvp_1vp_1.$$

TABLE 6

Progeny of the Cross: $\frac{A_1A_2CR\ Vp_1}{A_1A_2cr\ vp_1} \times \frac{A_1A_2Cr\ vp_1}{A_1A_2Cr\ vp_1}$

Non-viviparous		Viviparous	
Purple	Non-purple	Purple	Non-purple
1387	1373	0	2742*

* 459 dilutely colored seeds are included in this number.

If the homozygous viviparous phenotype inhibits color formation and is independent of any of the color genes, a ratio of one colored seed to three colorless seeds with no colored viviparous seeds is expected. Table 6 shows that this is the case.

The suppression of color formation in viviparous seeds could explain the linkage indication with R reported by Eyster (1931b) and which this study has failed to confirm.

An occasional viviparous seed is found in the progeny of the above cross which is very dilutely colored. No analogous class of seeds is found among the normal seeds on the same ear. Plants from such seeds produce ears which are homozygous for vivipary and have predominantly colorless seeds with an occasional seed that is faintly colored. Such faintly colored seeds could result from the failure of the viviparous gene to completely suppress color formation.

GENETIC STUDIES INVOLVING VIVIPAROUS$_2$

A stock of $\underline{vp}_2$ was obtained from Maize Genetics Coöperation. In this stock $\underline{vp}_2$ seeds are smaller than normal seeds on the same ear. It is not known if this reduced size is the result of the $\underline{vp}_2$ allele or of a second closely linked gene such as $\underline{re}_1$ or $\underline{re}_2$, as indicated by Eyster (1931a).

Eyster (1931a, b, 1935) reported linkage of this gene with $\underline{Pr}$, $\underline{Re}_1$, $\underline{Re}_2$, $\underline{Sc}_1$ and $\underline{Y}_2$ on the basis of F_2 data. The following map distances were calculated:

$\underline{Pr}$ - $\underline{Vp}_2$	20 - 40
$\underline{Re}_1$ - $\underline{Vp}_2$	15.5
$\underline{Re}_2$ - $\underline{Vp}_2$	0 - 1.2
$\underline{Sc}_1$ - $\underline{Vp}_2$	9.6
$\underline{Y}_2$ - $\underline{Vp}_2$	5.3

Burnham reported linkage between this mutant and Pr of 23% and between Bt_1 and vp_2 of 9% as the result of F_2 studies (1935). These data indicate that the gene is on chromosome five.

To check these values a plant heterozygous for vp_2 and Pr was out-crossed to a Pr tester. Plants from 31 of the red seeds and 30 of the purple seeds resulting from this cross were self-pollinated and the resulting ears checked for vp_2 seeds to determine the constitution of the gametes contributing pr or Pr with regard to vp_2. The results of this backcross can be seen in Table 7.

TABLE 7

Backcross Progeny of $\frac{pr+}{pr+} \times \frac{+ vp_2}{pr +}$

Parental combinations		Recombinations		Total	% recombination
+ vp	pr +	+ +	pr vp		
18	25	12	6	61	29.5

GENETIC STUDIES INVOLVING VIVIPAROUS$_5$

Seeds of this mutant were generously furnished by Dr. Burnham. Tests for alleleism made between this mutant and a phenotypically similar one furnished by Dr. Sprague revealed that the two mutants were alleles.

Occasionally seeds of this mutant will fail to germinate. The exact conditions responsible for preventing germination are not known.

Plants heterozygous for viviparous$_5$ and homozygous for yellow endosperm pollinated by pollen of B-1b plants produce three classes of seeds: (1) yellow viviparous, (2) yellow dormant and (3) white dormant. These results can be explained if the normal allele of $\underline{vp}_5$ is located in the section of the short arm of chromosome one attached to the B centromere. If the hyperploid sperm fertilizes the polar fusion nucleus and the deficient sperm the egg nucleus of a female gametophyte which carries $\underline{vp}_5$, class one seeds will result. If the reverse combination takes place, class two seeds will result. Class three seeds are produced by similar nuclear fusions as described for classes one and two, but the female gametes carry the normal allele for vivipary.

Pollen from B-1b plants has been used on forty-nine plants of families which are expected to yield fifty percent heterozygous $\underline{vp}_5$ plants. Of these forty-nine crosses nineteen have produced the three classes of seeds listed above. White non-viviparous seeds from the above pollinations should be heterozygous for $\underline{vp}_5$. Seven such plants were tested and all segregated vivipary.

In order to locate the position of this mutant on the short arm of chromosome one, crosses were made to translocation 1-9$_a$, which is linked to $\underline{wx}$. A plant heterozygous for $\underline{vp}_5$, $\underline{wx}$ and translocation 1-9$_a$ was crossed to a standard. Plants from the seeds of this cross were grown and the pollen checked for sterility. These plants

were then self-pollinated and the resulting ears classified for $\underline{vp}_5$. The results of this backcross can be seen in Table 8.

TABLE 8

Backcross Progeny of $\frac{wx\ T\ +}{+\ +\ vp_5} \times \frac{+++}{+++}$

Parental Recombinations		Recombinations						Total	% Recombination	
		Region 1		Region 2		Region 1,2				
wxT+	++vp	wx+vp	+T+	wxTvp	+++	wx++	+Tvp		Reg.1	Reg.2
32	38	5	0	17	14	0	1	107	5.6	29.9

These data indicate that the gene is located thirty units from the translocation. Since the translocation break is only .17 of the cytological distance from the centromere on the short arm of chromosome one, it is probable that $\underline{vp}_5$ is distal to this break point.

The crosses of this mutant with B-1b reveal some information as to the mechanism involved in producing vivipary. If only the seeds resulting from female gametophytes carrying the viviparous allele are considered two classes of seeds can be recognized with regard to the genotype of the embryo and endosperm. These are given in Table 9.

TABLE 9

Endosperm and Embryo Genotypes Resulting from the Pollination of vp_5 Gametophytes by B-1b Pollen

	CLASS 1 Gametic Unions Egg - Hyperploid sperm Polar nuc.-Deficient sperm	CLASS 2 Gametic Unions Egg - Deficient sperm Polar nuc.-Hyperploid sperm
Embryo	vp VP VP	vp
Endo-sperm	vp vp	vp vp Vp Vp

These two classes of seeds should be useful in determining the separate roles of the endosperm and embryo, if any, in inducing premature germination. If premature germination is produced by changes only in the endosperm, then seeds of class one should be viviparous. If instead the viviparous gene is active only in the embryo in promoting premature germination, then class two seeds should be viviparous. Only seeds of class two are observed to germinate prematurely.

Sprague (1936) found a similar situation with $viviparous_7$ mutants. As the result of hetero-fertilization he obtained seeds which had normal embryos with genetically viviparous endosperms and also seeds with normal endosperms which had embryos that were genetically viviparous. As in the case of $viviparous_5$, only the seeds with genetically viviparous embryos germinated prematurely.

As will be shown below, the situation is the same in viviparous$_8$.

These results indicate that vivipary is determined by the genotype of the embryo and is independent of the genotype of the endosperm.

GENETIC STUDIES INVOLVING VIVIPAROUS$_7$

This mutant is characterized by pink endosperm and pink scutellum. The seedlings produced are also pink and lack chlorophyll when germinated in the light, but have a faint bluish-green color if germinated in darkness. Practically all seeds of this mutant germinate prematurely.

Nine different mutants of this type, including the one reported by Sprague (1936), were grown and intercrossed to test for allelism. These tests indicated that all nine are allelic.

Dr. Sprague (unpublished) had indicated the probable linkage relations of this gene to be $\underline{vp}_7$ - $\underline{pr}$ - $\underline{gl}_8$. A plant homozygous for the genes responsible for aleurone color and heterozygous for $\underline{vp}_7$, $\underline{pr}$ and $\underline{gl}_8$ was crossed to a plant homozygous for $\underline{gl}_8$. The ears resulting from this cross had only purple seeds. These seeds were planted and 192 of the seedlings were classified with regard to $\underline{gl}_8$. At maturity the glossy and normal plants were self-pollinated and the mature ears classified for

$\underline{vp}_7$ and $\underline{pr}$. The results of the backcross are seen in Table 10.

TABLE 10

Backcross Progeny of $\frac{vp_7\ pr\ gl_8}{+\ +\ +} \times \frac{+\ +\ gl_8}{+\ +\ gl_8}$

Parental Combinations		Recombinations						Total	% Recombination	
		Region 1		Region 2		Region 1,2			Reg.1	Reg.2
79	82	9	19	2	0	0	0	192	14.6	1.0

Since the position of $\underline{gl}_8$ on chromosome five with respect to $\underline{pr}$ is unknown, it is impossible to tell the direction of this linkage map with reference to the centromere. Crosses to locate $\underline{vp}_7$ with respect to $\underline{bm}_1$ and translocation $5\text{-}9_a$, the break point of which is located .80 of the distance from the centromere on the long arm of chromosome five, have been started and should give an answer to this question.

GENETIC STUDIES INVOLVING VIVIPAROUS$_8$

Viviparous$_8$ originally appeared in stocks derived from seed exposed at the Bikini atom bomb test. Seeds of this mutant have yellow endosperms and produce green seedlings which, if grown to maturity, produce dwarf plants.

One plant heterozygous for $\underline{vp}_8$, pollinated by pollen from a B-la plant, produced an ear segregating viviparous seeds. Such a pollination produces ears segregating large and small seeds. The large seeds are produced when the hyperploid sperm fuses with the polar nuclei and the deficient sperm fertilizes the egg nucleus, while small seeds result from the reverse combination. Upon close examination of the two types of seeds produced by this pollination, it was found that part of the large seeds were viviparous, while none of the small seeds germinated prematurely. Since only large seeds germinate prematurely, it appears that the genetic constitution of the embryo determines premature germination. Sixteen known heterozygous plants pollinated by B-la pollen all segregated for vivipary. In addition, thirty-six crosses were made to plants, half of which should be segregating $\underline{vp}_8$. Of these, 18 segregated for $\underline{vp}_8$. These data establish that $\underline{vp}_8$ is located in the long arm of chromosome one.

To locate this gene more exactly, a cross was made to translocation 1-7c carrying the genes $\underline{br}$ and $\underline{bm}_2$. Plants from seeds of this cross were checked for pollen sterility and the semisterile plants self-pollinated to determine if they were heterozygous for $\underline{vp}_8$, and also outcrossed to $\underline{br}$, $\underline{bm}_2$ or $\underline{br}$ $\underline{bm}_2$ plants. Three outcrosses were grown and the plants classified for $\underline{br}$, $\underline{bm}_2$ and pollen semisterility. Self-pollinations were made to

test for $\underline{vp}_8$. One backcross involved only the br gene, another only $\underline{bm}_2$, while the third involved both br and $\underline{bm}_2$. The results are summarized in Table 11. The translocation is very close to the locus br. The position of $\underline{vp}_8$ was found to be br-32.4 - $\underline{vp}_8$-13.3 - $\underline{bm}_2$.

TABLE 11

Backcross Progeny Involving T 1-7_c, br, vp_8 and bm_2

Genotype of F_1 Parent	Parental Combination		Recombinations Region 1*		Region 2		Region 1,2		Total	% Recombination Reg.1	% Recombination Reg.2
$\frac{Tbr+bm}{++vp+}$	9	10	4	8	5	1	0	1	38	34.2	18.4
$\frac{Tbr+}{++vp}$	7	8	1	6	-	-	-	-	22	31.8	
$\frac{T+bm}{+vp+}$	11	17	3	5	3	0	0	1	45	31.1	8.9
Total Region 1			13	19			0	2	105	32.4	
Total Region 2					8	1	0	2	83		13.3

* A total of 175 plants were classified for the translocation and br without obtaining a crossover between them, therefore, in this summary the regions T-vp and Tbr-vp are both considered region 1.

GENETIC STUDIES INVOLVING VIVIPAROUS$_9$

Viviparous$_9$ originally appeared in stocks derived from seed exposed to an X-ray dosage of 20,000 r-units. This mutant is phenotypically similar to $\underline{vp_5}$, but is more strongly viviparous. Intercrosses have shown that they are not allelic.

Pollinations made with A-B interchange stocks gave no indications that the locus of this gene is in any of the regions involved in these interchanges.

Table 12 gives F_2 data from ears heterozygous for $\underline{vp_9}$ and $\underline{wx}$. These data do not deviate significantly from a 9:3:3:1 ratio expected for two independent genes. The chi square value for the observed ratio is 4.677 which is expected about 20 percent of the time. A stock which was homozygous for $\underline{wx}$ and heterozygous for $\underline{vp_9}$ was crossed to translocation 7-9$_{A76}$. Seven F_2 ears which segregated $\underline{wx}$ and $\underline{vp_9}$ were obtained. The resulting ratios are presented in Table 13. The observed ratio differs very significantly from 9:3:3:1.

Three plants from a family in which two-thirds of the plants were expected to be heterozygous for $\underline{vp_9}$ and two plants from a family in which one-half the plants were expected to be heterozygous from $\underline{vp_9}$ were pollinated by B-7b pollen. None of these produced $\underline{vp_9}$ seeds. The probability that only non-viviparous plants were chosen by chance in making these pollinations is 1/108. There-

fore it is improbable that the gene is located distal to the translocation in the long arm of chromosome seven. Since ra and gl_1 loci are in the long arm of chromosome seven within the region translocated to the B centromere, this would indicate that the vp_9 locus is to the left of these genes. Crosses have been made to $glossy_1$ to determine the distance between vp_9 and this gene.

TABLE 12

F_2 Progenies of plants Heterozygous for wx and vp_9

Plant	Phenotypes				Total
	++	+wx	vp+	vp wx	
769-4	197	54	67	16	
769-8	152	48	34	11	
769-14	253	89	100	22	
Total	602	191	201	49	1043

TABLE 13

F_2 Progenies of Plants of the Genotype $\frac{+\ T\ +}{wx\ +\ vp_9}$

Plant	Phenotypes				Total
	++	+wx	vp+	vp wx	
1659-9	124	19	14	33	
1659-12	101	27	21	40	
1659-20	96	50	34	38	
1699-2	102	25	20	13	
1699-7	76	15	13	17	
1699-13	91	12	20	15	
1699-16	86	9	13	14	
Total	676	157	135	170	1138

EMBRYO CULTURE AS A TOOL FOR INVESTIGATING VIVIPARY

La Rue (1936) and Haagen-Smit et al. (1945) have successfully cultured corn embryos on chemically defined media. La Rue using embryos which had attained a scutellar diameter of 0.6 mm. or greater obtained good growth on a medium of salts and sugar solidified with 1% agar. Haagen-Smit et al., with a medium of salts and sugar plus some added growth factors, obtained growth of maize embryos greater than 0.3 mm.

La Rue (1936) reports that embryos placed upon the culture medium cease all embryonic growth and proceed

to grow at once into a seedling. Similar observations have been made with embryos of other plants, thus providing a practical way to overcome dormancy imposed upon embryos by some constituent of the environment within the seed coat.

Since maize embryos are potentially capable of germinating at a very early stage if removed from the seed, there must be some mechanism within the seed for inhibiting germination and prolonging embryonic growth. It is the alteration of this mechanism by the action of the viviparous gene which is responsible for producing prematurely germinating seeds.

Numerous germination inhibitors have been described in the literature. Konis (1940), Evenari (1949) and others have suggested that these substances are responsible for preventing precocious germination of developing embryos.

Most of the tests for the presence of germination inhibitors have been made on mature seeds, and thus the effect of the inhibitor upon the developing embryo is not known. On the other hand, when a substance is found which inhibits germination of the maturing embryo, it is difficult to determine if this substance is the actual substance utilized by the developing seed for inhibiting premature germination.

Genetic studies with maize have shown that some factors responsible for vivipary are inherited in a Mendelian manner. Crosses of $\underline{vp}_5$ with B-1b pollen have revealed that premature germination is dependent upon the genotype of the embryo. If dormancy of the developing embryo is caused by the presence of a germination inhibitor, the genetic evidence would suggest that viviparous embryos germinate prematurely because they are capable of germination in the presence of this inhibitor. Parallel test for germination inhibition can be made with viviparous and normal embryos. If an inhibitor is responsible for preventing premature germination, it should be inhibitory to normal embryos and not to viviparous. Embryo culture experiments were used to test for such an inhibitor.

In the following experiments only immature embryos that had not started to germinate were used. Viviparous_5 embryos were used predominantly in these studies because of the ease with which potentially viviparous and normal seeds could be separated at an early age on the basis of endosperm color.

The two culture media used in these experiments were La Rue's (1936) medium number 1 and Haagen-Smit's (1945). Twenty cc. of medium were added to 25 x 1500 mm. pyrex test tubes and autoclaved twenty minutes at fifteen pounds pressure and 250° F.

The seeds were cut from the cob as aseptically as possible and the kernels were then sterilized by one of two methods as follows: (1) placed in 70% ethanol for three minutes and then rinsed in sterile distilled water or (2) placed in a solution of one part zephiran chloride in 5,000 parts water for twenty minutes, after which time they were rinsed in sterile distilled water. Both of these methods proved effective in controlling contamination.

After cutting a window in the pericarp, the embryos were removed from the endosperm, measured and placed on the surface of the agar. The culture tubes were kept in the light at room temperature for the duration of the experiment. Embryos cultured in the dark responded the same as those cultured in the light.

A. Tests for a Germination Inhibitor in the Endosperm

Since embryos removed from the environment of the seed germinated when placed on the culture medium, the possibility of some inhibiting substance being present in the endosperm was investigated. To test this, three experiments were set up as follows: (1) Twenty embryos, 1 mm. in length, were placed on the surface of the culture medium along with pieces of endosperm, (2) A block of endosperm containing the intact embryo, 5 mm. in length, was removed from ten seeds and placed on the surface of the culture medium, and (3) A window was cut in the peri-

carp of ten seeds exposing the embryos, 5 mm. in length, but leaving them in place in the seed. These seeds were then placed on the surface of the culture medium. In the control experiments the embryos were placed directly on the agar.

Embryos placed on the agar surface with accompanying pieces of endosperm germinated as readily as the controls. The embryos exposed in place in the seed and those situated on a block of endosperm germinated at a slower rate than the controls. The initial growth of the root and plumule was delayed about twenty-four hours as compared with the controls. This difference is reflected in the plumule growth of embryos that have been cultured seven days. Table 14 shows that the mean plumule length of the controls is roughly twice that of the embryos left in contact with the endosperm. This delay could be the result of the endosperm drying on exposure to air or of the different nutritional environments of the control embryos and those on endosperm. As soon as the roots from embryos in experiments 2 and 3 penetrate the culture medium, the plumules grow at a rate which parallels that of the controls.

TABLE 14

Plumule Growth of Embryos in Contact with the Endosperm

	Controls	Embryos exposed in place	Embryos exposed on a block of endosperm
Mean plumule length in cm.	.84±.24		.39±.07
	.39±.09	.20±.04	

To determine if the above delayed effect was the result of an inhibitor of premature germination rather than an environmental effect, normal and viviparous embryos were exposed but left in place in the seed. These were then placed under conditions favorable for germination. No significant difference in germination rates of the two classes of embryos was observed. These experiments suggest that the endosperm does not contain a substance inhibitory to the germination of exposed embryos.

A volatile inhibitor may be present in the endosperm which is rapidly lost on exposure to the air. To test for such a substance, immature endosperms were quickly frozen after removing the embryos and were then lyophilized. The "lyophilizate" was collected without thawing and stored in a deep-freeze unit. The embryos were also frozen and lyophilized and the "lyophilizate" collected. It was anticipated that any volatile substances from the materials being lyophilized would be collected

along with the water in the "lyophilizate".

Petri dishes (60 x 15 mm.) were prepared with nutrient medium on which were placed five embryos. A few cc. of frozen "lyophilizate" were placed in a small vessel within the petri dish, following which the dishes were immediately sealed in petroleum jelly and kept at room temperature. A control was set up in which only water was placed in the enclosed vessel.

If present, a volatile inhibitor should be liberated when the "lyophilizate" melted, forming an inhibiting atmosphere in the sealed dish.

Neither embryos nor endosperm "lyophilizate" produced any inhibitory effect on the germination of the embryos.

B. The Effect of O_2 Tension on Germination

Various workers have reported that the impermeability of the seed coat to oxygen will inhibit the germination of embryos of some species, which, if removed from the confines of the seed coat are capable of germination (Atwood, 1914; Harrington, 1923; Spaeth, 1932; Stier, 1937; Crocker, 1906). Frequently such seeds can be induced to germinate if placed in an atmosphere of 100% oxygen. This suggests that enough oxygen for germination is capable of diffusing through the seed coat if the oxygen tension is raised high enough. In maize the pericarp and the underlying layers might act to

inhibit the diffusion of sufficient oxygen to the developing embryo to permit germination. An experiment was designed to test this.

Two 250 mm. desiccators were sterilized and used as culture chambers. In each were seven sterile 60 x 15 mm. petri dishes containing moist filter paper on which four seeds were placed. Two classes of seeds were used: (1) whole seeds with intact pericarps, and (2) seeds with a window of pericarp removed directly above the embryo without removing any of the underlying tissues between the pericarp and the embryo.

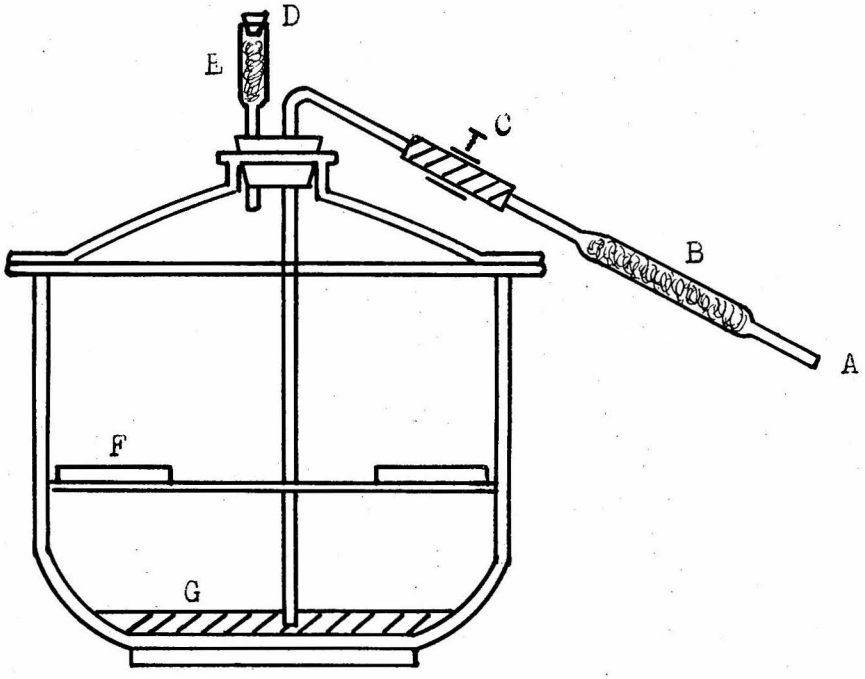
In each desiccator the following number and kinds of seeds were placed:

1. Four normal seeds with intact pericarps.
2. Eight $viviparous_5$ seeds with intact pericarps.
3. Eight normal seeds with a window in the pericarp.
4. Eight $viviparous_5$ seeds with a window in the pericarp.

Water was placed in the bottom of each desiccator to ensure a saturated atmosphere. The desiccators were then equipped as is shown in Figure 3 to permit the enclosed atmosphere to be changed. After the seeds were placed in the desiccators one desiccator was flushed with oxygen to produce an 100% oxygen atmosphere while the other retained an atmosphere of air. The gas within the desiccators was changed once every twenty-four hours over a

period of seven days. At the end of this time they were opened and the plumules were measured to determine the extent to which germination had proceeded. The results of this experiment can be seen in Table 15. Among the intact seeds germination of viviparous embryos was somewhat more advanced than normals under conditions of atmospheric oxygen. In a pure oxygen atmosphere the amount of germination of the viviparous seeds almost doubled, whereas, the germination of normal seeds remained the same. When a window of pericarp was removed above the embryo, the amount of germination increased in both classes of seeds. These results can be explained by making two assumptions: (1) Viviparous embryos are capable of germinating at a lower oxygen tension and are more sensitive to changes in oxygen concentration than normals, and (2) the pericarp is an efficient barrier to the diffusion of oxygen. Under these conditions the intact seed is expected to have a reduced oxygen tension within the pericarp. This reduced oxygen concentration may be sufficient to permit some germination of the viviparous embryos, but not of the normals. When the amount of oxygen available to the embryos is increased by raising the oxygen concentration, then the viviparous embryos may be sufficiently sensitive to respond to this increase, while the increase is not sufficient to stimulate the normal embryos to germinate. If, however, the pericarp is removed, then sufficient

FIGURE 3



A. Gas Inlet
B. Cotton Filter
C. Clamp
D. Gas Outlet
E. Cotton Filter
F. Petri Dish
G. Water

A desiccator as equipped for embryo culture studies involving gas mixtures.

oxygen under atmospheric conditions may reach the embryos to stimulate germination of both viviparous and normal embryos.

TABLE 15

Effect of Increasing the O_2 Tension and Removing the Pericarp Upon the Germination of Normal and Viviparous Embryos

	Air				100% O_2			
	Pericarp intact		No pericarp		Pericarp intact		No pericarp	
	+	vp	+	vp	+	vp	+	vp
Mean plumule length in cm.	.07 ±.09	.62 ±.21	.91 ±.37	2.69 ±.12	.07 ±.08	1.4 ±.20	.50 ±.04	.59 ±.12
Sig. of difference between the means	+		+		+		-	

It would appear that extremely high concentrations of oxygen are toxic to both normal and viviparous embryos. This inhibitory effect of high oxygen concentrations was observed in several other experiments. If viviparous embryos germinate prematurely because they are capable of germination at lower oxygen tension, there should be an oxygen concentration which would permit viviparous embryos to germinate but not normals.

Embryos of immature normal and viviparous seeds were exposed completely but left in place on the endosperm. These seeds were arranged in the desiccator as explained above for the previous experiment. In each desiccator

there were four dishes holding a total of sixteen normal seeds and four with sixteen viviparous seeds. After the seeds were sealed in, the desiccators were flushed out with the desired gas mixture. This was repeated once every twenty-four hours for six days. The above procedure was used in all of the following experiments of this section.

The gas mixtures were obtained by displacing water from five gallon glass bottles. By displacing this gas mixture with water the gas was then forced into the desiccators. This method of mixing gases was considered adequate for preliminary tests for differential effects of reduced oxygen. If such effects are found, more refined techniques could be used for mixing and supplying the gas. The following concentrations of oxygen were used: 20%, 2%, 1%, ½% and 0%. The balance of the gas was nitrogen. The results of these experiments are summarized in Table 16.

TABLE 16

Effect of Various Oxygen Tensions on the Germination of Viviparous and Normal Embryos

% O_2	0		½		1		2		20	
	+	vp	+	vp	+	vp	+	vp	+	vp
Expt. 71										
No. of Embryos	14	14			15	13	14	14	13	11
Mean plumule length in cm.	0	0			.13 ±.06	.20 ±.03	.63 ±.10	.78 ±.18	1.15 ±.12	1.22 ±.21
Sig. of difference between the means	-				-		-		-	
Expt. 72										
No. of Embryos	16	16	11	9	6	14			12	12
Mean plumule length in cm.	0	0	.64 ±.15	.70 ±.22	.87 ±.32	1.19 ±.14			1.4 ±.26	1.4 ±.18
Sig. of difference between the means	-		-		-				-	
Expt. 73										
No. of Embryos					16	16			15	16
Mean plumule length in cm.					1.39 ±.16	1.84 ±.16			2.08 ±.26	1.98 ±.23
Sig. of difference between the means					±				-	

At each oxygen concentration tested the growth of normal and viviparous embryos does not differ significantly. However, at oxygen concentrations of 2% or lower the viviparous embryos consistently exhibit more germination than normals. This suggests that viviparous embryos are able to germinate somewhat better than normals at these lower oxygen tensions. However, no oxygen concentration tested permitted viviparous embryos to germinate while preventing the germination of normals. Thus a difference in oxygen tension may have a minor effect, but is not adequate to explain the difference between the germination of normal and viviparous embryos.

C. Carbon Dioxide as an Inhibitor of Germination

The effect of carbon dioxide was studied, since it might reach high concentrations if the pericarp were impermeable. Normal and viviparous embryos were supplied with mixtures of air and carbon dioxide in desiccators. The following percentages of carbon dioxide were used: 25%, 50%, 99% and 100%. The results of these experiments are shown in Table 17.

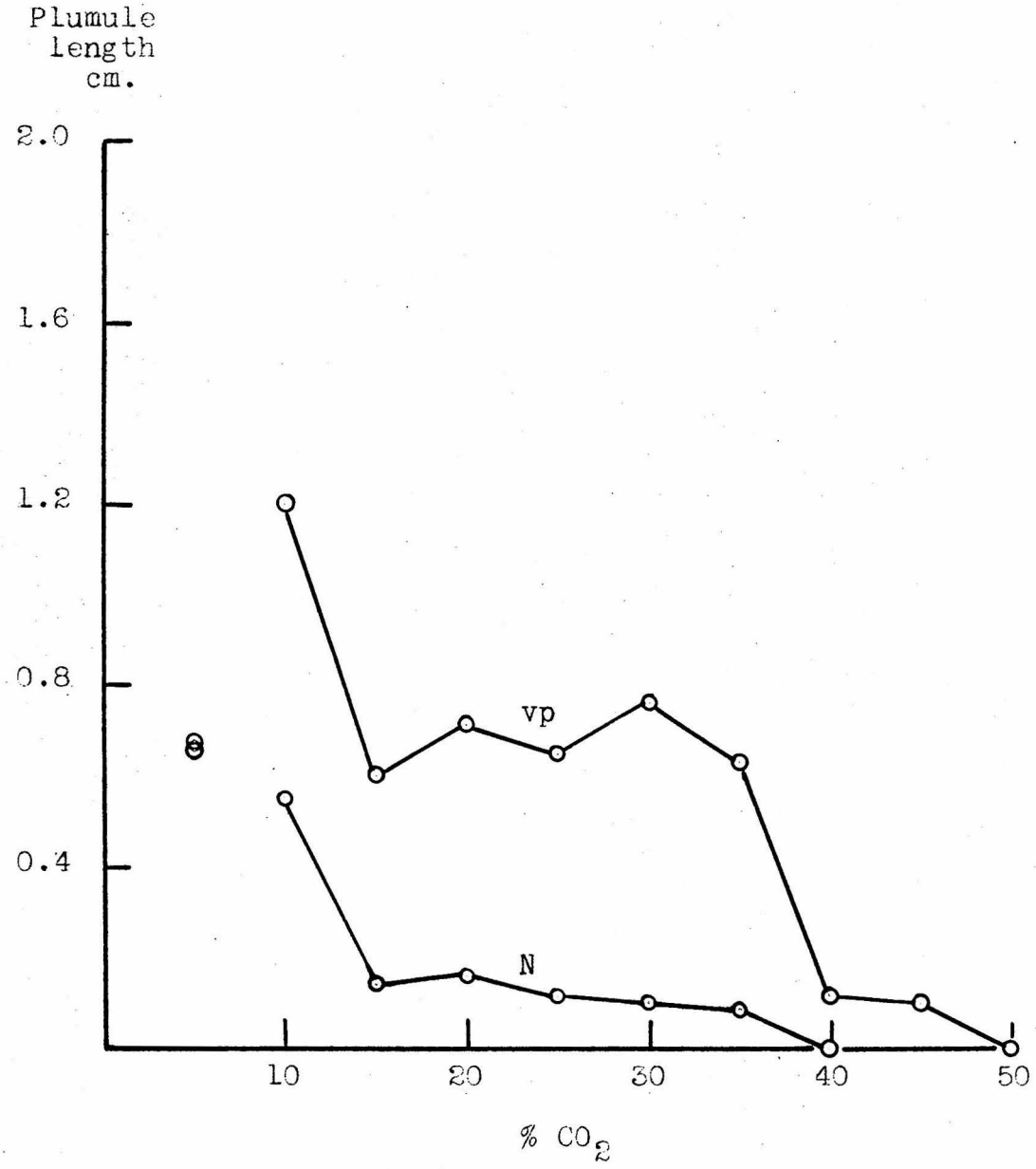
TABLE 17

/Effect of Various CO_2 Concentrations on the Germination of Viviparous and Normal Embryos

% CO_2	0		25		50		99		100	
	+	vp	+	vp	+	vp	+	vp	+	vp
No. of Embryos	16	16	18	9	13	14	16	16	16	12
Mean plumule length in cm.	1.47 ±.14	2.17 ±.14	.18 ±.04	.39 ±.10			0	0	0	0
Sig. of difference between the means	+		±							

Germination is inhibited by high concentrations of carbon dioxide. At the lower concentrations where germination is not entirely inhibited, viviparous embryos show more growth than normals. However, in air (without added CO_2) the growth of viviparous embryos also exceeds that of normals. More precise tests were made using concentrations of 5, 10, 15, 20, 25, 30, 35, 45, and 50 percent carbon dioxide in nitrogen with a standard oxygen tension of 1 percent. Fresh immature seeds were used with the first five concentrations of carbon dioxide. For the three high concentrations, mature seeds were used as plants in the field were killed by frost. The response of embryos from these latter seeds seems to be comparable with those obtained from the fresh material so the data have been included

Figure 4



Plumule length of normal and viviparous embryos as influenced by CO_2 (1% O_2 used in all experiments)

here. The results of these experiments are plotted in Figure 4.

In carbon dioxide concentrations of more than 10%, the growth of both normal and viviparous embryos is less than in air, showing an inhibiting effect of carbon dioxide. The growth of normal and viviparous embryos is inhibited differentially in concentrations of from 10 to 45%. Normal embryos are inhibited more strongly than are the viviparous. This differential effect is shown over a rather wide range of concentrations.

For carbon dioxide to control germination during the early stages of development, it must inhibit the growth processes which lead to germination, without seriously handicapping the growth of the embryo itself. Increase in scutellum size may be used as an indication of effect upon the embryo. Throughout all tests, there was a measurable increase in scutellum length in both normal and viviparous embryos.

In the oxygen tests, germination was observed in concentrations of 5% or more (Table 18). The growth of the plumule increased with increasing oxygen tension. This is also true, but to a lesser degree, of the growth of the scutellum (Table 18). Increasing oxygen tension increases both scutellar and plumule growth. There appears to be no differential effect on embryonic growth and germination.

TABLE 18

Change in the length of the scutellum as affected by the oxygen concentration

% O_2	0	½	1	2	20
Expt. 71					
No. of Embryos	27		28	18	23
Mean change in scutellum length (mm.)	.63		.6	1.05	1.34
Expt. 72					
No. of Embryos	32	22	20		24
Mean change in scutellum length (mm.)	.61	1.00	.84		1.15

Table 19 contains data from tests in which the embryos were sup lied sufficient oxygen but failed to germinate because of the carbon dioxide concentrations. In all but one case, the scutella enlarged as much as those grown under atmospheric conditions.

TABLE 19

Change in Length of Scutellum as Affected by CO_2 Compared with the Change in Air

Genotype	+		vp		+		vp	
	Air	1% O_2 99% CO_2	Air	1% O_2 99% CO_2	Air	10% O_2 50% CO_2	Air	10% O_2 50% CO_2
No. of embryos	15	16	16	16	16	13	16	14
Mean change in scut. length mm.	1.8 ±.14	1.4 ±.07	1.2 ±.18	1.4 ±.09	1.0 ±.09	1.08 ±.1	.69 ±.08	1.5 ±.08
Sig. of difference between the means		+		-		-		+

The above experiments indicate that viviparous$_5$ embryos are able to germinate at higher concentrations of carbon dioxide than are normal embryos, and that embryonic growth continues at concentrations of carbon dioxide which inhibit germination of both normal and viviparous embryos.

MISCELLANEOUS EXPERIMENTS BEARING ON THE NATURE OF VIVIPARY

A. The Role of the Cob in Inhibiting Premature Germination

The work of Oppenheimer (1922a, b) with inhibitors of germination found in the fruits of tomatoes and other plants suggested to Mangelsdorf (1926) that the cob of maize might contribute an inhibitor of germination. He observed that if mature seeds are left on the cob, but kept in a moist atmosphere by wrapping the ear in moist cotton, no germination resulted. Seeds of the same age but kept in a moist germinator showed signs of germination after ten days. Attempts to duplicate his experiment were not successful. In two such experiments germinating seeds were found on the ear and among those which were excised. As an additional test, a corn cob was ground in a Waring Blendor, filtered and the raw filtrate added to the culture medium on which were placed excised embryos. All ten embryos used in this experiment germinated after five days.

B. Acetaldehyde as a Possible Germination Inhibitor

Mazé (1910) suggested that acetaldehyde in the maturing seeds was responsible for preventing the embryo from germinating. The seeds upon drying lost their acetaldehyde and were, therefore, able to germinate.

To test the effect of acetaldehyde, normal embryos were cultured on slants of culture medium. Different concentrations and different amounts of acetaldehyde

solutions were added at the side of the slant and the tubes turned in a manner which prevented the acetaldehyde solution from touching the embryos. All tubes were corked to prevent the acetaldehyde from escaping.

Preliminary tests revealed that 0.15 cc. of a dilution of 1 part acetaldehyde in 6 parts of water was near the threshold for inhibiting germination when applied in this manner. One such experiment with ten normal embryos produced the results shown in Table 20.

TABLE 20

Effect of Acetaldehyde on the Germination of Excised Maize Embryos (see text for details)

Germination after seven days with AA.		Germination five days after transferring to medium minus AA.	
No. Germ.	No. Not Germ.	No. Germ.	No. not Germ.
4	6	5	5

In all of the tests a given amount of acetaldehyde either did not prevent germination or had a lethal effect. The embryos classified as showing germination in Table 20 did not germinate in the normal manner and appeared rather sickly. In five days after transfer to an acetaldehyde free medium, there was very little growth, indicating that acetaldehyde is detrimental to embryos even at concentrations that will allow germination.

Viviparous and normal embryos from the same ear were tested for a difference in response to the acetaldehyde concentration used above. Ten immature embryos (3-4 mm. in length) from $\underline{vp}_5$ seeds and ten normal embryos (3-4.5 mm. in length) were cultured under the same conditions as reported in the previous experiment. After thirteen days there were no indications of germination in any of the viviparous embryos. Five of the embryos from normal seeds showed varying amounts of germination. Similar experiments with $\underline{vp}_4$ embryos showed no significant difference in the response of these embryos and their controls to acetaldehyde. Normal and viviparous embryos do not differ significantly in their response to acetaldehyde.

Plants carring the $\underline{vp}_5$ allele pollinated by B-1b interchange pollen produce two classes of seeds in which the embryo and endosperm differ with respect to the gene for vivipary. The genotypes of these two classes of seeds are given in Table 9, pg. 38. If vivipary is the result of the endosperm producing less acetaldehyde, seeds of Class 1 (Table 9) should be viviparous, but this is not the case. The Class 2 seeds (Table 9) are viviparous, while the Class 1 seeds are dormant.

The data obtained from genetic and embryo-culture experiments indicate that acetaldehyde is not responsible for preventing premature germination.

C. Auxin Analyses of Viviparous Mutants

Popoff (1941) suggested a correlation between the auxin content of developing kernels of some of the small grains and their ability to sprout in the heads. Since it is known that the developing corn seed is a rich source of indole acetic acid (auxin) (Avery, 1935; Stehsel, 1949), a change in the auxin level might be responsible for inducing the embryo to germinate prematurely.

Viviparous and normal seeds from the segregating ears of $\underline{vp_5}$, $\underline{vp_7}$, or $\underline{vp_9}$ were analyzed. These mutants were used because of the ease with which the immature viviparous seeds can be separated from normal seeds on the ear. The kernels were cut from the ear and frozen. The frozen material was lyophilized and ground. This material was analyzed for the total ether extractable auxin.

To extract the auxin the sample was hydrolyzed at pH 9 at 100° C. for a half-hour. After cooling, the pH was adjusted to pH 3 and the auxin extracted with five washes of ether. The intensity of the color produced by Salkowski's reagent was used as an indication of the amount of auxin present. This intensity was read on a Klett-Summerson colorimeter, using a 540 mμ filter. The results are summarized in Table 21.

TABLE 21

Total Auxin Content of Normal and Mutant Seeds from Ears Segregating for vp_5, vp_7, and vp_9

Plant	Mutant	Pheno-type	Auxin γ/gm. dry wt.	Auxin γ/seed	Condition of Dormancy
5801-17	vp_5		92.8	3.80	dormant
-17		vp	92.8	3.70	dormant
-9			254.0	25.8	dormant
-9		vp	242.6	22.02	dormant
-13			142.4	18.0	dormant
-13		vp	150.0	18.0	germinating
-31			163.2	20.0	dormant
-31		vp	154.9	17.4	germinating
5803-28	vp_7		79.6	2.9	dormant
-28		vp	76.7	2.6	dormant
-35			68.2	5.8	dormant
-35		vp	60.5	4.8	dormant
-17			94.2	11.4	dormant
-17		vp	95.2	10.7	dormant
-39			80.5	11.4	dormant
-39		vp	74.0	10.6	germinating
5802-26	vp_9		91.0	3.2	dormant
-26		vp	80.9	2.8	dormant
-1			50.4	4.1	dormant
-1		vp	42.4	3.3	dormant
-14			68.0	5.9	dormant
-14		vp	60.1	4.9	dormant
-10			60.2	7.7	dormant
-10		vp	49.5	6.3	germinating

The auxin content of vp_5 or vp_7 seeds does not differ from that found in normal seeds from the same ears when compared on a per gram or per kernel basis. Viviparous$_9$ seeds consistently yielded less auxin than normal kernels when analysed on a per gram or a per kernel basis. The differences in total auxin between viviparous and

normal seeds of the first two mutants do not indicate that total auxin *per se* is responsible for vivipary. However, the consistently lower values found in vp_9 seeds suggest a possible relationship between vivipary and auxin content in this mutant.

It should be pointed out, however, that the method of extraction does not differentiate between free, physiologically active and bound non-active auxin since both of these are extracted under the conditions used. A more critical test would be to assay for free auxin in normal and viviparous seeds. Because the amount of free auxin is relatively small, this analysis would require a bio-assay which has not been done.

Since it is the embryo which is responsible for producing vivipary, a more critical test would be an analysis of its auxin content. Preliminary tests of viviparous and normal embryos have failed due to the low level of auxin and the presence of large amounts of oils which interfered with auxin extractions.

DISCUSSION

Within a seed the developing embryo is located in an environment which is peculiarly suited for supplying the requirements for its germination. Studies with immature maize embryos have revealed that they are potentially capable of germinating soon after pollination (about ten days). However, germination does not normally occur at this early date in spite of such an apparently favorable environment. Instead of germinating the embryo continues to grow in a manner which is typified by differentiation of leaf primordia, enlargement of these primordia and a parallel increase in the size of the scutellum. Embryonic growth is further marked by an absence of cell elongation.

Several premature germination mechanisms have been postulated by workers in the field of plant dormancy. Kidd (1914) suggested that carbon dioxide, present in the tissue of developing pea and bean embryos was responsible for preventing their germination in the pod. However, Harriet Bonner (unpublished), also working with peas, found immature embryos contained a leachable inhibitor of germination which was thought to be responsible for maintaining the developing embryo in a dormant state. Pope (1949), working with barley, was able to induce vivipary in this plant by keeping the germ moist. He proposed that the immature embryo does not germinate because it is

unable to obtain enough water due to anatomical barriers which are changed upon ripening. James Bonner (unpublished), working with tomatoes found that the osmotic pressure of the juice was sufficient to prevent germination. A viviparous strain of tomatoes was also studied in which the osmotic pressure was somewhat below that required for the inhibition of germination. Konis (1940) also found osmotic pressure of tomato juice to be inhibitory. Oppenheimer (1932a, b), working with tomatoes and other plants, was able to obtain substances from the fruits of these plants which inhibited seed germination. Reinhard (1933) and Konis (1940) confirmed Oppenheimer's observations on tomatoes. Randolph and Cox (1943) reported that the endosperm of Iris seeds contains an inhibitor which prevents the germination of the embryo. Many other workers have found similar germination inhibitors in the fruits and seeds of a large number of plants.

Evanari (1949) has published an excellent review of the work done in this field. Various workers have speculated on the role of these germination inhibitors in preventing premature germination of developing seeds. However, there seems to be a lack of critical experiments indicating that these substances are actually responsible for this phenomenon.

Inherited changes which permit the developing seed or embryo to germinate in an environment which normally

induces dormancy, enable one to study this mechanism in a more critical manner.

Different factors may be responsible for inhibiting this process at different stages in ontogeny of the seed. As the seed matures it passes through a period which is optimum for germination; a period in which the internal environment of the seed is similar to that found during normal germination. This is the most critical period for the prevention of germination. The mutations studied in this report all appear to disrupt the normal inhibiting mechanism during this critical period and as a consequence, premature germination results. The response of viviparous and normal embryos to carbon dioxide suggest that this gas might play a role in preventing germination at this critical time. It must be pointed out, however, that the tolerance of viviparous embryos for high concentrations of carbon dioxide is due to some mechanism as yet unknown.

A mechanism involving carbon dioxide which explains vivipary can be suggested. If, as the seed matures, its enclosing membranes become more permeable to carbon dioxide, then as the seed approaches maturity its internal environment of carbon dioxide will decrease. Sprague (1936) measured the water content of developing seeds and found that it also decreased as the seed matured. This water loss terminated with maturity at which time there was not sufficient water within the seed to support ger-

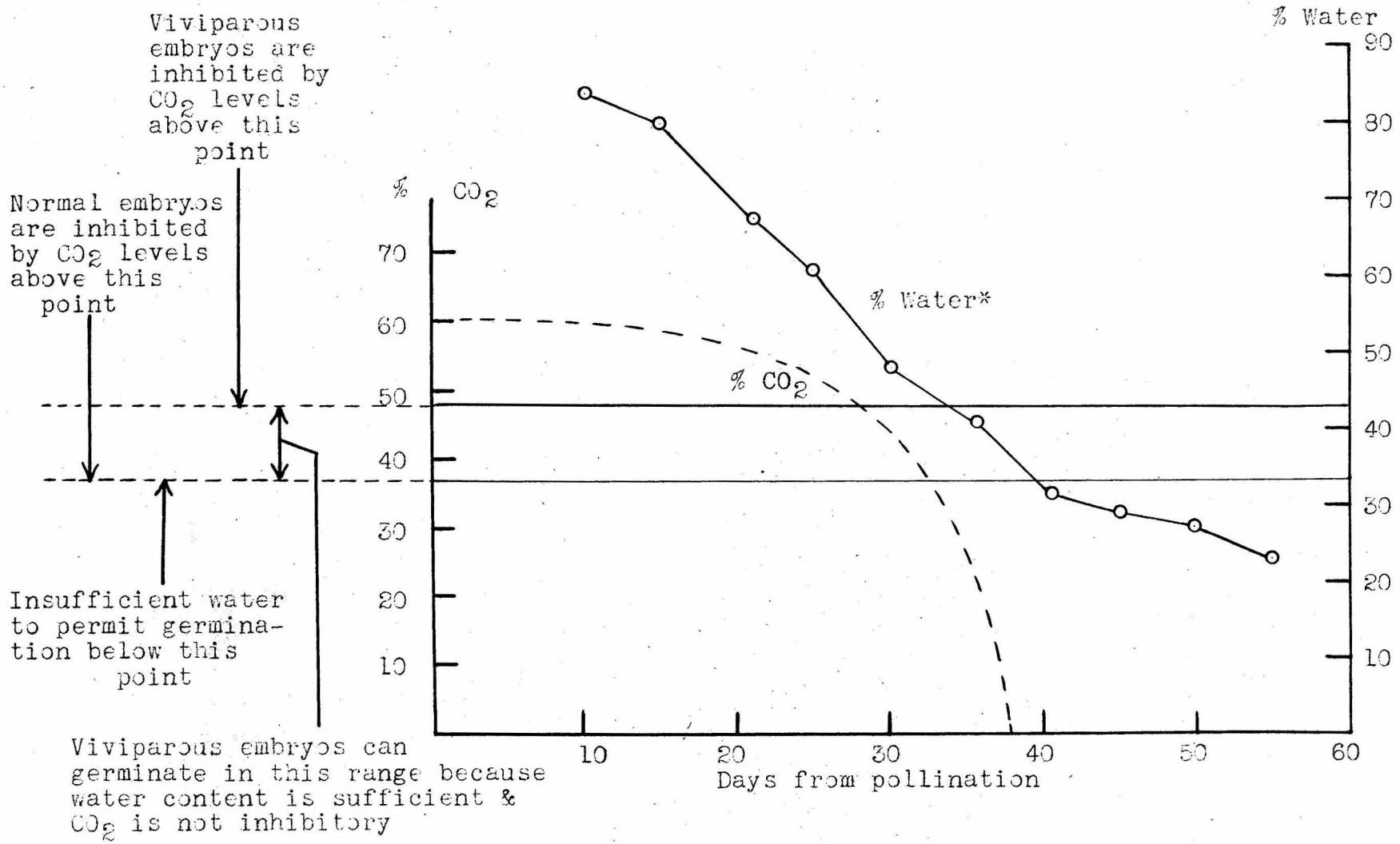
mination and the seed remained dormant. Viviparous$_1$ seeds at the time of germination upon the ear have a water content of 36.5±0.7 percent, which is comparable to the value of 38.0±0.81 percent found by Sprague (1936) for mature seeds at the time of germination.

We have, then, this picture of a maturing seed with regard to its carbon dioxide and water relations. As the seed matures, there is a parallel decrease of both water content and internal carbon dioxide tension. While the water level is high, the carbon dioxide tension of the seed is sufficient to prevent germination. As the seed matures, its lowered carbon dioxide tension would permit germination, but the water content is too low.

On this interpretation, the carbon dioxide concentration of the seed should be about 40-45% at the time other conditions are favorable for germination, therefore, normal embryos cannot germinate, but viviparous ones will be able to do so because of their increased carbon dioxide tolerance. This mechanism can be schematically represented as in Figure 5.

There are at least six different mutations that interrupt the mechanism for preventing premature germination. Tests have not been made with the other viviparous mutants to determine if they have a tolerance for carbon dioxide similar to that exhibited by $\underline{vp}_5$ embryos.

FIGURE 5



A schematic presentation of how the water and CO_2 relationships of a maturing seed could permit the expression of vivipary

* From Sprague, 1936, pg. 347

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