Characterization of the Two Maize Embryo-Lethal Defective Kernel Mutants
\textit{rgh*}-1210 and \textit{fl*}-1253B: Effects on Embryo and Gametophyte Development

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\textbf{ABSTRACT}

We have examined the effects on embryonic and gametophytic development of two nonallelic defective-kernel mutants of maize. Earlier studies indicated that both mutants are abnormal in embryonic morphogenesis as well as in the formation of their endosperm. Mutant \textit{rgh*}-1210 embryos depart from the normal embryogenic pathway at the proembryo and transition stage, by developing meristematic lobes and losing bilateral symmetry. They continue growth as irregular cell masses that enlarge and become necrotic. Somatic embryos arising in \textit{rgh*}-1210 callus cultures display the \textit{rgh*}-1210 mutant phenotype. Mutant \textit{fl*}-1253B embryos are variably blocked from the coleoptilar stage through stage 2. Following formation of the shoot apex in the mutant embryos, the leaf primordia and tissues surrounding the embryonic axis continue growth and cell division, while the scutellum ceases development and becomes hypertrophied. Mutant \textit{fl*}-1253B embryos are unable to germinate, either in mutant kernels or as immature embryos in culture, and the mutant scutellar tissue does not produce regenerable callus. Expression of the \textit{fl*}-1253B locus during male gametophytic development is revealed by a marked reduction in pollen transmission as a result of mutant expression during the interval between meiosis and the initiation of pollen tube growth. In both mutants, there is considerable proliferation of the aleurone cells of the endosperm. Mutant expression of \textit{rgh*}-1210 in the female gametophyte is revealed by the abnormal antipodal cells of the embryo sac. These results show that these two gene loci play unique and crucial roles in normal morphogenesis of the embryo. In addition, it is evident that both mutants are pleiotropic in affecting the development of the endosperm and gametophyte as well as the embryo. These pleiotropisms suggest some commonality in the gene regulation of development in these three tissues.

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HE \textit{defective kernel} (\textit{dek}) mutants of maize are single-gene recessive mutants that are defective in both endosperm and embryo. Many are embryo lethals in that they fail to germinate at kernel maturity. Mutants of this type are common both as spontaneous occurrences (Manglesdorf 1923, 1926) and in mutagenized populations (Sprague 1941; Neuffer and Sheridan 1980; Dolfini et al. 1985). These mutants can provide insight into the role of genes in embryogenesis (Sheridan and Clark 1987a).

Embryogenesis in maize results in the formation of an embryo consisting of a massive cotyledonary structure, the scutellum, and a well-differentiated miniature plant, the embryonic axis. It occurs in two distinct phases: an initial period of morphogenesis in which all of the tissue types and kinds of structures of the mature embryo are established, followed by a period of structural elaboration and storage product deposition (Sheridan and Neuffer 1981).

A group of embryo-lethal \textit{dek} mutants blocked prior to the formation of leaf primordia has been recovered from a collection of ethyl methane sulfonate induced mutants (Sheridan and Neuffer 1982). These mutants become blocked at various stages during the initial morphogenesis phase of embryo development, that is from the proembryo stage through stage 1 (according to the staging scheme of Abee and Stein 1954; see Figure 1). Most of the mutants are stage-specific in their blocks. They vary in their degree of morphological abnormality and in their phenotype at kernel maturity (Sheridan and Neuffer 1982). These mutants are being characterized in detail genetically and developmentally (Clark and Sheridan 1986; Sheridan and Thorstenson 1986; Sheridan and Clark 1987a).

We report here on two mutants from this group, \textit{rgh*}-1210 and \textit{fl*}-1253B. Both mutants are included in this group because examination of mature mutant kernels in fresh dissection revealed that \textit{rgh*}-1210 was blocked stage specifically at the transition stage, while \textit{fl*}-1253B appeared to be blocked variably from the transition to the coleoptilar stage (Sheridan and Neuffer 1982). Embryos of both mutants are morphologically abnormal, as a result of proliferation of...
Embryonic tissues. In earlier studies, both were found to be lethal at kernel maturity, and neither responded to culture on basal or enriched media during an auxotroph screen (Neuffer and Sheridan 1980; Sheridan and Neuffer 1980). Their chromosome arm location is unknown although they have been screened by use of a set of B-A translocations involving all of the chromosome arms except for 6S, 7S, and 8S (Neuffer et al. 1986). Mutant fl*-1253B displays a deficiency of mutant kernels on segregating self-pollinated ears, indicating that the mutant gene is expressed in the gametophyte.

We have continued the detailed characterization of these two mutants because they appear to represent gene loci that control developmental processes essential for normal morphogenesis of the embryo (Sheridan and Clark 1987a). In this paper we report that the mutants rgh*-1210 and fl*-1253B are non-allelic loci. Each is involved in the maintenance of structural organization within the embryo, although at different stages of development. Both genes affect the development and normal function of the embryo, the endosperm, and the gametophyte.

**MATERIALS AND METHODS**

**Mutants and maintenance of stocks:** The mutants are referred to by their laboratory designations, indicating their endosperm phenotypes at kernel maturity. Mutant rgh*-1210 has a rough crown; fl*-1253B has a floury endosperm. Endosperm development in both mutants is quite extensive; mutant kernels of both are only slightly smaller than normal kernels on the same ear. Mutant kernels are first recognizable on segregating self-pollinated ears at 13–16 days after pollination. Because of their lethality, the mutants are maintained as heterozygotes in strains either derived from the composite Aho or our early maturing strain ES478LF.

Materials for this study were either field grown in Columbia, Missouri (kindly provided by M. G. Neuffer), or field or greenhouse grown in Grand Forks, North Dakota. Nomenclature and pedigree procedures are those of Neuffer (1982).

**Allelism tests:** Allelism was assessed by means of a complementation test in which a cross was made between two plants, using the double pollination technique described by Sheridan and Clark (1987b).

**Developmental studies:** In order to determine the time and stage of onset of the mutant phenotype, the pattern of development in mutant embryos, and the variability of mutant expression, we examined mutant and normal embryos by paraffin sectioning and by scanning electron microscopy of whole mounts at intervals following pollination. Seven to ten mutant embryos were examined in each sample. Histological and scanning electron microscopic techniques used were those described in Clark and Sheridan (1986) except that some sectioned material was stained with Toluidine Blue O following removal of paraffin (Sakai 1978).

**Developmental stages:** The classification scheme of Abbe and Stein (1954), based on the stage of shoot apex development, was used in this study (see Figure 1). Five intervals of kernel development were distinguished: very early (4–16 days), early (16–25 days), mid (25–35 days), late (35–45 days), and kernel maturity (65 day or older).

**Embryo culture:** When placed into culture, immature normal maize embryos respond either by precocious germination of the embryonic axis, or by producing callus from the scutellum. By manipulating the hormone levels in the medium, one or the other response may be obtained. Mutant embryos of rgh*-1210 and fl*-1253B were cultured in two kinds of experiments. In rescue experiments, mutant embryos were cultured on basal and hormone supplemented media in an attempt to obtain continued embryonic development or germination; in callus induction experiments, mutant embryos were cultured on auxin-containing media to test their capacity to undergo callus formation and regeneration by organogenesis or somatic embryogenesis. Mutant embryos were cultured on MS medium (Murashige and Skoog 1962) and on N6 medium (Chu et al. 1975). In some cases media were supplemented with proline and abscisic acid (ABA), since the addition of proline and ABA has been reported to improve the growth of cultured embryos (Norstog 1979) and the frequency of somatic embryogenesis in maize embryo cultures (Green, Armstrong and Anderson 1983). In the rescue experiments, mutant embryos were placed with their scutellar surface against the medium on plates containing 50 ml of medium. Embryos were cultured on basal media supplemented with 0.001, 0.01, and 0.1 mg/l ABA, 1 mg/l naphthylacetic acid (NAA), 0.03 mg/l kinetin, or 0.1 mg/l gibberellic acid (GA), as well as the combination of 1 mg/l NAA, 0.05 mg/l kinetin, and 0.1 mg/l GA. In the callus induction experiments, mutant embryos were placed on their embryonic axis against the medium on plates containing 50 ml of MS or N6 media supplemented with 0.75–2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). Cultures were maintained at 25° under 16 hr of low intensity light alternating with 8 hr of darkness, and were subcultured as needed. All media variants were used to culture normal embryos at the coleoptilar stage or stage 1, and were observed to support embryonic development and germination or, in the case of the 2,4-D supplemented media, callus formation.

**Germination tests:** Since histological and SEM observations established that rgh*–1210 and fl*–1253B mutant embryos were capable of forming leaf primordia, sandbench germination tests were conducted using a large number of mutant kernels. One hundred mutant kernels were planted from a total of two segregating ears (75 from one ear, 25 from another). Five normal segregating ears were planted from each ear as controls. Germination was scored after 21 days.

**Transmission studies:** Transmission of the mutant fl*-1253B allele through the pollen and through the egg was tested in reciprocal crosses between a color marked normal stock and a colorless stock carrying the mutant allele, by double pollination (Sheridan and Clark 1987b). In the next generation test families consisting of colored kernels from ears produced by the reciprocal crosses were planted, and the plants were self-pollinated to reveal the presence of the mutant. In the families where the mutant stock had served as female in the reciprocal cross, the proportion of plants producing ears that segregated for the mutant represents the rate of transmission of the mutant allele through the egg. Likewise in families where the mutant stock had served as pollen parent, the proportion of segregating ears represents the frequency of transmission of the mutant allele through the pollen. Deviation from expected ratios was assessed by a replicated goodness-of-fit test yielding $\chi^2$ values based on log-likelihood ratios. Impairment of pollen germination was distinguished from impairment of pollen tube growth by comparing the proportion of mutant kernels in the top 1/3 to that in the bottom 1/3 of segregating self-pollinated ears from the test families (Bianchi and Loren-
an attempt to divide the ears into two sharply defined high and low transmission groups, based on their contribution to the heterogeneity in maize. These statistical procedures are described in Sokal and Rohlf (1981).

**Allelism**

Crosses between the two mutants produced only normal kernels; they therefore are not allelic. In direct tests both rgh*-1210 and fl*-1253B were also shown to be non-allelic to the unlocated developmental mutants previously described, bmo*-747B and cp*-1418 (Sheridan and Thorstenson 1986). They are almost certainly also non-allelic to the located developmental mutants dek22 (IL), dek23 (2L) (Clark and Sheridan 1986) and dek31 (4L) (Sheridan and Thorstenson 1986; Sheridan and Clark 1987), inasmuch as neither rgh*-1210 and fl*-1253B is uncovered by the B-A translocation stocks which uncover these mutants (Sheridan and Neuffer 1986; Neuffer et al. 1986).

**Developmental studies**

Both rgh*-1210 and fl*-1253B are pleiotropic in their expression. Below we briefly describe features of the development of the embryo, the endosperm, and the gametophyte generation that are relevant to presenting the results of our analyses of these two mutants.

**Morphogenesis of the maize embryo:** Normal maize embryogenesis has been described in detail (Randolph 1984; Kieselbach 1949; Abe and Stein 1954). The two fundamental regions of the embryo, the embryo proper and suspensor, are blocked out during the proembryo stage (4-8 dap). Division of the embryo proper into embryonic axis and scutellum occurs at the transition stage (8-10 dap) with the differentiation of a region of meristematic cells at the eventual site of the shoot apex (Figure 1), while the remainder of the embryo proper begins its enlargement to form the scutellum. At the beginning of the coleoptilar stage (10-12 dap) the coleoptile makes its appearance as a bulge on the surface of the scutellum surrounding the shoot apex. During this stage and the following one, the scutellum increases greatly in size by cell division and enlargement, and a vascular system differentiates, linking the embryonic axis and the scutellum. The embryonic shoot-root axis itself becomes evident with the formation of the root apical meristem deep within the embryo. This new axis is established at an angle to the axis of the transition stage embryo. During stage 1 (12-14 dap) the first of the six leaf primordia that differentiate during embryogenesis appears at the base of the shoot apical meristem and grows upward across the face of the shoot apex. By the end of stage 1, deposition of starch and storage proteins is well underway. By stage 2 (14-16 dap), when the second leaf primordium differentiates, the scutellum is opaque and its cells have taken on a distinctly parenchymatous appearance.

**Endosperm:** The mature maize kernel (caryopsis) contains the embryo and a massive endosperm. The endosperm is genetically identical to the embryo, except that it includes an additional copy of the genome contributed by the female parent. However its developmental fate is very different than that of the embryo. The endosperm may be divided morphologically and functionally into three regions: an upper storage portion; a lower (chalazal) region which apparently functions to transfer nutrients to the developing embryo and endosperm (Schell, Kiessling and von Lammeren 1984); and a distinct single-celled outer layer, the aleurone. Its morphology is similar to the epidermis of the scutellum. The aleurone is functionally distinct from the remainder of the endosperm in participating in the hormonal control of germination.

**Male and female gametophyte:** The mature female gametophyte consists of the embryo sac containing eight or more haploid cells derived by mitosis of the megaspore. Although these cells are genetically identical, they differ in morphology and function: The egg and central cell are double fertilized to become embryo and endosperm respectively; the two synergid cells adjacent to the egg function during fertilization and then degenerate; and the three antipodal cells at the distal end of the embryo sac undergo several divisions and persist within the embryo sac until they

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**RESULTS**

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are crushed by the rapidly expanding endosperm. The function of the antipodals is unknown.

The mature male gametophyte consists of the pollen grain and pollen tube; it contains three genetically identical haploid nuclei. One, the vegetative nucleus, directs the formation and growth of the pollen tube but does not participate in fertilization. The other two nuclei (sperm nuclei) are condensed and functionally inert during pollen germination and pollen tube growth, but function in double fertilization.

**Very early development of rgh*-1210:** Embryos of this mutant first became retarded during early embryogenesis by 8 dap, and in most cases were blocked subsequently at the transition stage (Table 1). Abnormal proliferation of embryonic tissues from 10 dap onward was evidenced by the irregular shape of the embryos and their production of meristematic lobes (Table 1). Abnormality was also observed in two of the three other constituents of the embryo sac: the antipodal cells showed unusual enlargement and altered patterns of growth at 8 dap, and the aleurone layer of the endosperm began proliferating at 10dap, about the same time as the embryo evidenced morphological abnormality.

**Development of rgh*-1210 from 16 dap until kernel maturity:** At early kernel development (Figure 2, a–c) normal embryos had reached stage 4, with a length of 11 mm. Most mutant embryos were at an abnormal transition stage, and averaged 0.94 mm in length, although one embryo had formed a rudimentary coleoptilar ring. Lobes of tissue protruded from the surface of the embryos. Regions of small densely staining meristematic cells were present in all mutant embryos, and were especially evident in the lobes (compare Figure 2b with the normal transition stage embryo in Figure 1). In face view it is apparent that the embryos lacked bilateral symmetry (Figure 2c).

By mid kernel development (Figure 2, d–f) normal embryos had reached stage 5 (Figure 2d) and were at least 10 mm in length. Mutant embryos averaged 1.03 mm in length. They remained at an abnormal transition to early coleoptilar stage. There was no evidence of histodifferentiation within the embryos, and cells within the embryo were enlarged and highly vacuolated (Figure 2e). Meristematic lobes had continued to form and overgrow another one another (Figure 2f).

By late kernel development (Figure 2, g–i) normal embryos were at stage 5 and were at least 10 mm in length. Mutant embryos had become disorganized cell masses of varying size, averaging 1.11 mm in length.

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**TABLE 1**

Developmental stages of embryos in sectioned kernels from ears segregating for rgh*-1210 examined at 4–16 dap

<table>
<thead>
<tr>
<th>Ear age (dap)</th>
<th>Normal embryos</th>
<th>Mutant embryos</th>
<th>retarded embryos/total embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotype</td>
<td>Length (mm)</td>
<td>Phenotype</td>
</tr>
<tr>
<td>4</td>
<td>Zygote-early proembryo</td>
<td>0.06 ± 0.01</td>
<td>Zygote-early proembryo</td>
</tr>
<tr>
<td>6</td>
<td>Proembryo</td>
<td>0.14 ± 0.01</td>
<td>Proembryo</td>
</tr>
<tr>
<td>8</td>
<td>Transition</td>
<td>0.30 ± 0.03</td>
<td>Transition</td>
</tr>
<tr>
<td>10</td>
<td>Coleoptilar</td>
<td>0.07 ± 0.11</td>
<td>Abnormal proembryo-transition; irregular shape; aleurone proliferating</td>
</tr>
<tr>
<td>12</td>
<td>Stage 1</td>
<td>1.11 ± 0.10</td>
<td>Abnormal transition; aleurone proliferating</td>
</tr>
<tr>
<td>14</td>
<td>Stage 1</td>
<td>1.63 ± 0.90</td>
<td>Abnormal transition-coleoptilar with lobes; aleurone proliferating</td>
</tr>
<tr>
<td>16</td>
<td>Stage 2</td>
<td>2.49 ± 0.11</td>
<td>Abnormal transition with lobes; aleurone proliferating</td>
</tr>
</tbody>
</table>

*Each ear used as a source of kernels for analysis was known to be segregating, but the genetic constitution of individual kernels was unknown. Therefore a sample of ten kernels would be expected to include one or more mutant kernels with P = 0.94. (Since these were self-pollinated ears a 3:1 ratio is expected and failure to find at least one mutant kernel in a sample of 10 = (0.75)^10 or 0.06.) Consequently at least ten kernels were sectioned unless two classes of embryos were identified in a smaller sample. Embryos having retarded or abnormal development were judged to be mutant embryos with a phenotype that clearly differed from normal embryos. The absence of two developmental classes of embryos was judged to indicate that the phenotype of mutant embryos did not yet differ from that of normal embryos.
Figure 2
Breakdown of cells was evident internally (Figure 2h); no organized features were evident on the surface of mutant embryos (Figure 2i).

At kernel maturity (Figure 2, j–l) normal embryos were at stage 6 (Figure 2j) and were at least 11 mm in length. Mutant embryos had continued to degenerate. They averaged 1.81 mm in length. Collapsing regions within the mutant embryos (Figure 2k) caused their outward appearance as knobby masses (Figure 2i). In fresh dissections, it could be seen that the embryos and the endosperm surrounding them were often discolored gray or black.

The aleurone, which is normally a smooth single cell layer, continued proliferative growth. It became several cells thick and progressively more convoluted, especially in the region of the mutant embryo (Figure 2, b, e, h, and k). The starchy part of the endosperm of rgh*-1210 kernels enlarged considerably and became starch-filled, but it did not reach the size of the endosperm of a normal kernel.

Development of f*-1253B from 16 dap until kernel maturity: Examination of very early embryogenesis (4–16 dap) was not undertaken for this mutant, because examination of sectioned materials showed that mutant embryos were smaller but morphologically normal when mutant kernels could first be distinguished on segregating self-pollinated ears. Therefore, the time when mutant embryos first become retarded as compared to their normal counterparts remains undetermined.

At early kernel development normal embryos were at stage 2 (Figure 3a) and a length of 5 mm. All the mutant embryos were at stage 1; their morphology was normal for their stage and they averaged 1.42 mm in length (Figure 3b). Mutant embryos examined slightly later in this period as whole mutants were at stage 2 (Figure 3c) and a length of 5 mm. The disparity of growth rates between these two regions of the embryo had not expanded (compare Figure 3c with the normal stage 1 embryo in Figure 1).

By mid-kernel development normal embryos had reached stage 4 (Figure 3d) and were 12 mm in length. Mutant embryos were at an abnormal stage 1 or 2 and averaged 3.16 mm in length. They all showed morphological abnormalities, and varied considerably in size. In sectioned materials it was evident that the cells of the scutellum were enlarged and vacuolated, while the lower part of the embryo around the embryonic axis had increased in size as the result of cell division (Figure 3e). In all embryos the scutellum was reduced in relative size compared to that of a normal stage 1 or 2 embryo, and the coleoptile and tissues of the region surrounding the embryonic axis appeared swollen (compare Figure 3f with the normal stage 1 and 2 embryos of Figure 1).

By late kernel development normal embryos were at stage 5 (Figure 3g), and a length of 12.8 mm. Mutant embryos remained at an abnormal stage 1–2 and averaged 1.82 mm in length. Hypertrophy of the scutellum and proliferation of tissue of the embryonic axis and surrounding regions was more pronounced than in the previous period (Figure 3h; also compare this embryo with the normal embryo in Figure 3a, which is at the same developmental stage). In some embryos proliferation of leaf primordia occurred (in Figure 3i the first leaf primordium is swollen and displaced to one side of the shoot apex). However in no case did the shoot apical meristem itself undergo proliferation.

At kernel maturity normal embryos were at stage 6 and a length of 14 mm (Figure 3j). Mutant embryos were all abnormal, and ranged from coleoptilar stage through stage 2. They averaged 2.47 mm in length. Tissues of the coleoptile and leaf primordia had proliferated and obscured the shoot apical meristem in the mutant embryos (Figure 3k). In the most advanced embryos, the degenerating scutellum consisted of a small outgrowth perched atop a massive, convoluted embryonic axis (Figure 3l). The disparity of growth rates between these two regions of the embryo had produced an internal distortion of the embryonic axis (Figure 3, k and l). Regions of necrosis and cell collapse were evident in several of the mutant embryos.

The aleurone layer of mutant f*-1253B endosperms proliferated into a convoluted multicelled layer. This proliferation was first seen at mid-kernel development, and became quite pronounced by kernel maturity (it is best seen in Figure 3e). The starchy part of mutant endosperms became large and starch filled. They differed from normal endosperm in having a floury texture.

Sandbench germination test of f*-1253B

All of the normal kernels germinated. None of the mutant kernels germinated, indicating that in mutant embryos, although the capacity to differentiate leaf primordia is present, the ability of those primordia to function normally, i.e., to germinate, is absent.

FIGURE 3.—Development of normal and mutant f*-1253B embryos at early (top row), middle (second row), and late kernel development (third row), and at kernel maturity (bottom row). In mutant embryos the scutellum ceases growth and development early in kernel development, while tissues surrounding the embryonic axis continue to grow and divide, and eventually overgrow other parts of the embryo (scale bar = 0.5 mm). (a) Normal embryo 13 dap, (b) mutant embryo 13 dap, (c) mutant embryo 16 dap, (d) normal embryo 25 dap, (e) mutant embryo 25 dap, (f) mutant embryo 29 dap, (g) normal embryo 45 dap, (h) mutant embryo 41 dap, (i) mutant embryo 50 dap, (j) normal embryo 69 dap, (k) mutant embryo 67 dap, and (l) mutant embryo mature kernel.
Embryo culture

*In vitro* germination of *rgh*-1210: No germination was observed among 32 mutant embryos cultured on basal MS or N6 media. This indicates that the block to continued embryonic growth and development suffered by mutant kernels *in vivo* could not be overcome by removing them from the kernel environment. Neither did the exogenous application of hormones rescue them from their mutant block; no germination occurred among 69 embryos cultured on hormone-supplemented media.

Callus production and somatic embryogenesis in *rgh*-1210: A total of 131 of 156 embryos cultured on auxin-supplemented media at early, mid-, and late kernel development produced callus. One embryo cultured at mid-kernel development and five cultured at late kernel development produced abundant friable embryogenic callus after five months in culture (Figure 4a). Callus from three of these embryos became actively embryogenic, and became covered with somatic embryos (Figure 4a). These somatic embryos displayed the same mutant phenotype as zygotic *rgh*-1210 embryos (Figure 4, b and c). When removed to basal media, the somatic embryos did not continue to proceed through the normal embryogenic sequence. Production of mutant somatic embryos in *rgh*-1210 demonstrates that the mutant defect is the result of the expression of the *rgh*-1210 gene in the embryo, and is neither a secondary effect produced by the mutant endosperm nor does it require the kernel environment for its expression.

*In vitro* germination of *fl*-1253B: None of 19 *fl*-1253B mutant embryos cultured at late kernel development on basal media germinated, despite the fact that most probably had differentiated leaf primordia. Likewise none of 27 mutant embryos cultured on hormone supplemented media germinated. As was the case with *rgh*-1210, mutant embryos of *fl*-1253B could not be rescued by either removal from the kernel environment, or by the application of exogenous hormones to the culture medium. This indicates that the block to germination caused by the mutant defect of *fl*-1253B is a result of the expression of the *fl*-1253B gene in the embryo, and is not a secondary result of the defective mutant endosperm.

Callus production in *fl*-1253B: Mutant embryos were cultured at early, mid, and late kernel development. Of 57 embryos cultured, 47 produced some form of callus. However this callus was derived from the embryonic axis rather than the scutellum, and was not of the type that supports regeneration by organogenesis or somatic embryogenesis.

Transmission of the mutant *fl*-1253B allele

Expression of the *fl*-1253B mutant allele in the gametophyte was indicated by a reduced sexual transmission of the mutant allele. This was evidenced by a deficiency of mutant kernels on self-pollinated ears of heterozygous plants, since an average of 13.6% mutant kernels were obtained instead of the expected 25%.

Rate of transmission through the egg and pollen: Transmission of the mutant allele was found to be normal through the egg (43.1%, the expected being 50%; Table 2), but significantly reduced through the
pollen (22.5% instead of the expected 50%).

**Distribution of mutant kernels on self-pollinated ears:** The basis of the \( f^{*}-1253B \) mutant defect in the male gametophyte was determined by examining the distribution of mutant kernels on segregating self-pollinated ears from the test families. The frequency of mutant kernels in the top 1/3 (14.5%) did not differ significantly from that in the bottom 1/3 (12.9%), indicating that the reduced rate of transmission of the \( f^{*}-1253B \) allele is the result of a reduced pollen germination rate, rather than reduced pollen tube growth of pollen carrying the mutant allele (Table 3).

**Segregation ratio on self-pollinated ears:** The percentage of mutant kernels on segregating self-pollinated ears was 13.6%, representing a 6:1 ratio (Table 3). However there was significant ear-to-ear variation in segregation ratio (note the heterogeneity \( \chi^2 \), Table 3). The proportion of mutant kernels on individual ears varied from 7.7% to 20.8%. One ear, E1814-5, did not differ from the expected 25% ratio. The STP grouping procedure failed to delineate nonoverlapping high and low transmission groups (Table 3).

**DISCUSSION**

The lack of narrow stage-specificity in the departure from the normal developmental pathway of these two mutants indicates that neither gene is required for a particular morphogenic event during embryogenesis. It seems rather than the normal function of each locus is needed to maintain the organized state of the embryo so that morphogenesis can continue, with the defect at the \( rgh^{*}-1210 \) locus producing an earlier and more profound effect on morphogenesis. Departure from normal embryo development not only occurs earlier in \( rgh^{*}-1210 \), but it also results in the disruption of the bilateral symmetry of the developing embryo and prevention of apical meristem formation, although cellular proliferation continues in the form of undifferentiated meristematic lobes. The mutant defect of \( f^{*}-1253B \), on the other hand, becomes apparent only after initiation of the shoot and root meristems. It affects specific parts of the embryo in different ways: the scutellum halts growth and its cells degenerate, but the region surrounding the embryonic axis as well as the leaf primordia continue growth and cell division. The fact that the shoot apex remains exempt from the deleterious changes occurring elsewhere in the embryo, along with the timing of the defect coinciding with shoot apex formation, leads to the speculation that a substance produced in this region may cause the disruption in structural organization of the \( f^{*}-1253B \) embryo.

The consistency in phenotype of each mutant, and particularly their differing courses of embryo development, indicate that the proliferative growth of the embryos is not a generalized disorganization in response to their failure to develop normally. This conclusion is supported by the observation that although the mutant \( dek22 \) is arrested very early in development at the transition stage and the mutant \( dek23 \) is blocked at an abnormal coleoptilar stage, neither undergoes abnormal tissue proliferation (CLARK and SHERIDAN 1986).
J. K. Clark and W. F. Sheridan

TABLE 3
Segregation in the top 1/2 and bottom 1/2 of self-pollinated ears of fl*-1253B

<table>
<thead>
<tr>
<th>Ear</th>
<th>Total kernels</th>
<th>%M*</th>
<th>Total kernels</th>
<th>%M</th>
<th>Total kernels</th>
<th>%M</th>
<th>χ² (25%M)</th>
<th>Low transmission group</th>
<th>High transmission group</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1818-3</td>
<td>125</td>
<td>9.6</td>
<td>174</td>
<td>6.3</td>
<td>299</td>
<td>7.7</td>
<td>60.398***</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>E1818-2</td>
<td>113</td>
<td>9.7</td>
<td>152</td>
<td>6.6</td>
<td>265</td>
<td>7.9</td>
<td>51.844***</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>E1818-1</td>
<td>165</td>
<td>9.7</td>
<td>211</td>
<td>8.1</td>
<td>376</td>
<td>8.8</td>
<td>65.246***</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>E1810-2</td>
<td>159</td>
<td>11.9</td>
<td>143</td>
<td>7.0</td>
<td>302</td>
<td>9.6</td>
<td>46.456***</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>E1810-3</td>
<td>125</td>
<td>9.6</td>
<td>123</td>
<td>9.8</td>
<td>248</td>
<td>9.7</td>
<td>37.727***</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>E1810-1</td>
<td>167</td>
<td>10.7</td>
<td>155</td>
<td>11.6</td>
<td>322</td>
<td>11.2</td>
<td>38.796***</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>E1811-2</td>
<td>149</td>
<td>13.4</td>
<td>131</td>
<td>9.9</td>
<td>280</td>
<td>11.8</td>
<td>30.535***</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>E1819-2</td>
<td>67</td>
<td>14.9</td>
<td>45</td>
<td>7.0</td>
<td>110</td>
<td>11.8</td>
<td>11.930***</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>E1814-3</td>
<td>88</td>
<td>13.6</td>
<td>96</td>
<td>12.5</td>
<td>184</td>
<td>13.0</td>
<td>16.110***</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>E1814-6</td>
<td>171</td>
<td>15.2</td>
<td>236</td>
<td>12.7</td>
<td>407</td>
<td>13.8</td>
<td>31.155***</td>
<td>10</td>
<td>9</td>
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<tr>
<td>E1814-4</td>
<td>147</td>
<td>16.3</td>
<td>146</td>
<td>12.5</td>
<td>295</td>
<td>14.3</td>
<td>20.025***</td>
<td>11</td>
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<tr>
<td>E1812-4</td>
<td>119</td>
<td>14.8</td>
<td>108</td>
<td>15.7</td>
<td>227</td>
<td>15.0</td>
<td>13.576***</td>
<td>7</td>
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<tr>
<td>E1814-8</td>
<td>106</td>
<td>17.9</td>
<td>213</td>
<td>13.5</td>
<td>319</td>
<td>16.3</td>
<td>14.125***</td>
<td>6</td>
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</tr>
<tr>
<td>E1811-1</td>
<td>189</td>
<td>19.0</td>
<td>155</td>
<td>15.5</td>
<td>344</td>
<td>17.4</td>
<td>11.355***</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>E1814-2</td>
<td>141</td>
<td>14.2</td>
<td>89</td>
<td>23.6</td>
<td>238</td>
<td>17.8</td>
<td>6.796**</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>E1814-4</td>
<td>80</td>
<td>17.5</td>
<td>120</td>
<td>19.2</td>
<td>200</td>
<td>18.5</td>
<td>4.813***</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>E1814-5</td>
<td>136</td>
<td>21.3</td>
<td>134</td>
<td>17.9</td>
<td>270</td>
<td>19.6</td>
<td>4.379*</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>E1814-5</td>
<td>140</td>
<td>21.4</td>
<td>172</td>
<td>20.3</td>
<td>312</td>
<td>20.8</td>
<td>3.007 NS</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Total 2387 14.5 2601 12.7 4988 13.6

Correlation between % segregating top vs. % segregating bottom: r = 0.72*

Do top and bottom differ in segregation ratio?
Observed % mutants

<table>
<thead>
<tr>
<th>F ratios:</th>
<th>Top 14.5%</th>
<th>Bottom 12.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between %M top and %M bottom</td>
<td>(d.f. = 1) F = 3.368 NS</td>
<td></td>
</tr>
<tr>
<td>Among ears</td>
<td>(d.f. = 17) F = 5.290*</td>
<td></td>
</tr>
</tbody>
</table>

Do ears differ from 3:1 ratio?
Observed ratio (top + bottom)
Pooled χ²
Heterogeneity χ²

397.196***
71.063***

* %M = percent mutant kernels.
** NS = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

The maize dek mutants are pleiotropic, a characteristic they share with many developmentally important mutants in other organisms (RAFF and KAUFMANN, 1983). Both rgh*-1210 and fl*-1253B are pleiotropic in that they affect embryo, endosperm, and gametophyte development. In both, mutant expression in the endosperm includes proliferation of the aleurone tissue. This proliferation is most noticeable in the vicinity of the embryo, suggesting that perhaps the developing aleurone is sensitive to a diffusible substance produced by the embryo. Abnormal thickening of the aleurone has been observed in other well-characterized maize embryo-lethal mutants in which the embryo undergoes disorganized growth, namely ptd*-1130 (dek31) and bno*-747B (SHERIDAN and THORSTENSON 1986), and the Germless-S mutant (SASS and SPRAGUE 1950). On the other hand aleurone proliferation does not occur in the two mutants which remain free of abnormal embryo proliferation, dek22 and dek23 (CLARK and SHERIDAN 1986). These data suggest that there may be a common mechanism for the maintenance of organization in the embryo and in the aleurone, and that the products of several gene loci are required for its proper functioning.

It has not been possible to address directly the question of whether the rgh*-1210 and fl*-1253B mutant embryo defects are themselves secondary pleiotropic effects of the mutant condition of the endosperm, since there are no B-A translocation stocks available that uncover these mutants (see SHERIDAN and NEUFFER 1982). However the production of mutant rgh*-1210 somatic embryos from mutant callus generated outside the kernel environment supports the notion that the embryonic defect is a result of the embryo's own genotype. The failure of excised fl*-1253B embryos to germinate when cultured indicates that the block to germination in this mutant also resides within the embryo itself, and therefore is specified by the embryo's own genotype.
Pleiotropy in both mutants extends to the gametophyte generation. In \textit{rgk*.-1210} the antipodal cells of the female gametophyte undergo abnormal enlargement and patterns of growth. Expression of the \textit{fl*.-1253B} mutation in the male gametophyte is revealed by a reduced sexual transmission of the mutant allele through the pollen.

Aberrant segregation ratios of embryo lethal mutants have been observed in Arabidopsis (Muller 1968; Meinke 1982) and maize (Wentz 1930). In some of these cases there was unequal distribution of mutant seeds (Muller 1968; Meinke 1982), indicating disturbances of pollen tube growth rather than reduced pollen germination. We infer the time of \textit{fl*.-1253B} mutant expression to be the post-meiotic period leading to pollen germination, rather than at meiosis or during pollen tube growth. We conclude this because on segregating self-pollinated ears the mutant kernels are evenly distributed along the length of the ear.

We recognize that the reduction in transmission of the mutant allele could be due to a closely linked gametophyte factor or another closely linked mutant with a detrimental effect on pollen development or germination causing reduction of transmission of the chromosome carrying it and the \textit{fl*.-1253B} mutant allele. With regard to the latter possibility, it is true that the ethyl methane sulfonate treatment that produced the \textit{fl*.-1253B} mutation may have also induced another mutant at a nearby locus that causes reduced pollen transmission. There is also the possibility that this linked locus and the \textit{fl*.-1253B} locus may be located in a centromere region inasmuch as the latter was not uncovered by the B-A translocation set. Although we do not believe that this situation is very likely, it is evident that the testing for recombinants among very large populations might reveal such linkage.

Gametophyte (Ga) factors have been described in maize [reviewed in Coe and Neuffer (1977) and Bianchi and Lorenzoni (1975)]; the current genetic map contains five Ga loci located on four different chromosomes (Coe, Hoisington and Neuffer 1984). These gametophyte factors are not likely to be the cause of the \textit{fl*.-1253B} reduced transmission, since their chromosome arm, locations would permit them to be uncovered by the B-A translocation stocks which have failed to uncover \textit{fl*.-1253B}. Furthermore, if linkage had been broken between a \textit{dek} mutant with normal transmission and a gametophyte factor that severely reduced transmission, a sharply distinct set of ears segregating in a 3:1 ratio would be expected in a large population. The STP grouping procedure failed to identify such a group. Therefore we interpret these results to indicate the gametophytic expression of the \textit{fl*.-1253B} gene itself.

The expression of both mutants in the embryonic endosperm and gametophyte generation is intriguing. It is evident that both gene loci play unique and crucial roles in normal morphogenesis of the embryo. The requirement of expression of their normal alleles for normal development of the other tissues suggests that the organism employs the same genes to control developmental processes in different tissues. The capacity of haploid gametophytes in cultured anthers and ovules to develop directly into embryos is consistent with the commonality in gene regulation of development in these different tissues.

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**LITERATURE CITED**


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