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# Three ENU-induced alleles of the murine quaking locus are recessive embryonic lethal mutations

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**Summary**

The quaking (*qk*) locus on mouse chromosome 17 has been defined by a single viable quaking allele. Three new alleles of quaking were selected after ENU mutagenesis by their failure to complement the quaking phenotype. The $qk^{k2}$ allele was induced on wild-type chromatin and the $qk^{kt1}$ and $qk^{kt4}$ alleles were induced on *t*-chromatin. Each is a recessive embryonic lethal mutation. They fail to complement each other and are not complemented by the deletion, $Tt^{Orl}$. Homozygotes and hemizygotes die at 8–9·5 days gestation, but not at a single precise time. Because the classical quaking mutation complements the lethality of these new alleles, but they fail to complement its quaking phenotype (myelination defect), we conclude that the quaking$^+$ function is required for embryonic survival as well as for myelination.

## 1. Introduction

The quaking (*qk*) locus on mouse chromosome 17 has been defined by a single allele whose properties include (1) a viable, fully recessive phenotype of seizures and quaking, (2) sterility in males due to defective spermatid differentiation (Bennett *et al.* 1971), (3) a severe deficiency of myelin in the central nervous system (Sidman *et al.* 1964), and (4) a less severe deficiency of myelin in the peripheral nervous system (Samorajski *et al.* 1970). These traits map to a single locus, so the genetic basis of these phenotypic effects appears to be a single mutation. The quaking phenotype is manifested in homozygotes at the onset of myelination, about 10 days after parturition, indicating that the wild-type quaking function is necessary for normal myelination.

Three new alleles of *qk* were induced by ethylnitrosourea (ENU) mutagenesis in this laboratory (Bode, 1984; Justice & Bode, 1986). Each allele was selected by its failure to complement the quaking phenotype. Thus, animals heterozygous for both the viable quaking allele and any of the ENU-induced $qk^k$ alleles exhibit the characteristic quaking and seizures of the original mutant (Bode, 1984; Justice & Bode, 1986). In contrast to the original *qk* allele, the $qk^k$ alleles all lack male sterility; hence, $qk^k/qk$ males are fully viable and fertile.

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Based on its action in *Drosophila*, ENU is expected mainly to cause single base changes, rather than rearrangements or deletions (Vogel & Natarjan, 1979). Where studied in the mouse, the reported changes are an AT–TA transversion and an AT–GC transition (Popp *et al.* 1983; Peters *et al.* 1985). Thus, mutations induced by ENU will probably identify properties of a single locus. Early experiments with the ENU-induced $qk^k$ alleles suggested that they were homozygous and hemizygous lethal. Therefore, to determine that the $qk^k$ alleles were embryonic lethal mutations and to characterize the time of lethality during development, pairwise complementation and embryological studies were undertaken. We present evidence here that none of the three ENU-induced *qk* alleles complements any other $qk^k$ allele, or the $Tt^{Orl}$ deficiency, to give viable, quaking animals. In addition, we show that complementation failure is caused by the early embryonic death of hemizygous, doubly heterozygous, or homozygous $qk^k$ embryos.

## 2. Materials and methods

### (i) *Mouse strains and mutants*

The viable *qk* mutant allele was obtained from Dr Eva Eicher, and is maintained heterozygous with $T+tf$ by brother–sister matings.

The $qk^k$ and $qk^{k2}$ isolates of the $qk^{k2}$ allele were induced by ENU mutagenesis of a (C57BL/6 × CBA/Ca)F1 male (Bode, 1984). Although they were isolated

from the same mutagenized male, and are probably identical alleles, each is maintained as a separate stock. The $qk^k$ isolate was 10 outcross generations away from the mutagenized background at the time of this study, and the $qk^{k2}$ isolate was 8 generations away from the mutagenized background. Each stock has varying contributions from the $t^{w5}$, C57BL/6 and CBA/Ca strains listed below. At the time of this study, $qk^k$ and $qk^{k2}$ had been backcrossed twice into the inbred CBA/Ca strain, but were being maintained as balanced lethals with $T+tf$ ($qk^k$) and $Tqktf$ ($qk^{k2}$).

The quaking alleles induced in the $t^{w5}$-haplotype, $qk^{kt1}$, $qk^{kt3}$, and $qk^{kt4}$ (Justice & Bode, 1986), are maintained as coisogenic strains by backcrossing into the $t^{w5}$ inbred strain mentioned below. The $qk^{kt3}$ and $qk^{kt4}$ isolates are probably identical alleles since they were isolated as litter-mates from the same mutagenized male, but are maintained as two separate stocks. $qk^{kt1}$ is at the sixth generation of backcross inbreeding, $N = 6$, $qk^{kt3}$ at $N = 5$ and $qk^{kt4}$ at $N = 6$. Each carries the $t^{w5}$ lethal that is a part of the $t^{w5}$ haplotype and each transmits the *t*-chromosome with segregation distortion from the male.

Mice carrying the $Tt^{Orl}$ mutation were obtained from Dr Lee Silver. This mutation has a 3 cM deletion that includes *T* and *qk*, but the mutant chromosome also has properties of a partial *t*-haplotype, hence the designation $Tt^{Orl}$ (Silver, Lukralle & Garrels, 1983). The strain is maintained in a CBA/Ca background and was at $N = 2$ at the time of this study.

The $T+tf/+t^{w5}+$ strain was obtained from Dr Michael Sherman, who in turn received it from Dr Dorothea Bennett. It had been inbred at Kansas State University by brother–sister mating for over twenty generations before these experiments.

The C57BL/6 (B6) and CBA/Ca (CBA) strains were obtained from The Jackson Laboratory, Bar Harbor, ME, and had been maintained in our colony by brother–sister mating for 7 years. The F1 hybrids used in this study were from a cross of C57BL/6 females with CBA/Ca males. The mice mated for embryological studies were offspring from a cross of this F1 hybrid generation with the parental *qk*, $t^{w5}$, or $Tt^{Orl}$ strain. All desired animals could be selected by their tail phenotype. For example, normal-tailed animals from crosses of $+/+ \times T++tf/+qk^{kt1}t^{w5}+$, $+/+ \times T++tf/+qk^{kt4}t^{w5}+$, and $+/+ \times Tqktf/+qk^{k2}+$ were chosen to mate to obtain embryos for dissection. Also, $Tt^{Orl}/+$ animals from the stock strain were mated to $+/+$ animals and short-tailed animals were selected for mating to obtain embryos for dissection.

The $tct^k$ (*t*-complex tail interaction) and $tf^k$ (tufted) mutations were induced by ENU mutagenesis of (C57BL/6 × CBA/Ca)F1 males (Bode, 1984). When heterozygous with Brachyury (*T*), *tct* mutations cause the mouse to be tailless. This strain is maintained in a B6 background by *inter-se* matings of homozygotes. The $tf^k$ mutation is a viable recessive mutation and is maintained by *inter-se* matings of homozygotes after backcrossing to $N = 9$ into the C57BL/6 inbred strain.

(ii) *Embryology*

Females were placed with the appropriate male in the late afternoon and checked for a vaginal plug the following morning. If no plug was observed on the first day, the mice were left together and examined each morning until a plug was detected. The day of the plug was designated day 0 of embryonic development. Pregnant mice were sacrificed by cervical dislocation, the uterus was removed and placed in phosphate buffered saline (PBS), pH 7·4. Embryos were removed by dissection and placed in fresh PBS for observation and photography. Embryos were staged by somite pair number and external characteristics. Stages given are those in Theiler (1972). If the embryo was being resorbed and somite number was not obtained, other characteristics were observed to give the approximate stage. Cases of extreme degeneration of embryonic tissue or empty implantation sites were classified as resorbed.

## 3. Results

(i) *The* $qk^k$ *mutations fail to complement in any combination*

Results of genetic crosses assessing complementation are summarized in Table 1. No viable quaking animals were obtained in intercrosses of $qk^k$ or $qk^{k2}$ heterozygotes, or in crosses of $qk^k$ heterozygotes with $qk^{k2}$ heterozygotes. In these crosses, any viable homozygous $qk^k$ or $qk^{k2}$ animals should be normal-tailed. Note that $T/+$ are short-tailed mice, $T/t^{w5}$ and $T/tct^k$ are tailless, $t^{w5}/+$ and $tct^k/+$ are normal-tailed, whereas $T/T$ and $t^{w5}/t^{w5}$ are lethal. Only two normal-tailed animals were observed in these crosses using *T* as a dominant balanced lethal, and were progeny tested to be recombinants. Similarly, no viable, quaking animals were observed in crosses of $qk^{k2}$ heterozygotes with $qk^{kt1}$, $qk^{kt3}$, or $qk^{kt4}$ heterozygotes. In many of these crosses, a balanced lethal system was not used, but no quaking animals were observed. In crosses of $+qk^{k2}/tct^k+ \times qk^{kt1}T^{kt1}t^{w5}/+++$, however, quaking animals could be identified by short-tails. Only one short-tailed animal was born in a total of 33 offspring and was probably a recombinant. In addition, no viable quaking animals were obtained from crosses of heterozygotes of the three quaking alleles with $Tt^{Orl}/+$. $Tt^{Orl}/qk$ animals are short-tailed and quaking, but males are sterile. Even though the homozygous lethality of the $qk^{kt1}$ and $qk^{kt4}$ alleles could not be assessed directly, because of the presence of the $t^{w5}$-lethal in the $t^{w5}$-haplotype, these alleles failed to complement the $qk^{k2}$ allele and failed to complement the $Tt^{Orl}$ deficiency.

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Table 1. *Complementation testing of* $qk^k$ *alleles*

(A) Litters from crosses segregating for homozygous or heterozygous $qk^k$ animals

| Parental genotypes | Tail phenotype of offspring | | | |
|---|---|---|---|---|
| Female × Male | Short T | Tailless | Normal[a] | Total |
| $Tqktf/+qk^k+ \times Tqktf/+qk^k+$ | 23[b] | — | 0 | 23 |
| $T+tf/+qk^k+ \times T+tf/+qk^k+$ | 29 | — | 1[c] | 30 |
| $T+tf/+qk^k+ \times Tqktf/+qk^{k2}+$ | 23 | — | 1[d] | 24 |
| $Tqktf/+qk^{k2}+ \times Tqktf/+qk^{k2}+$ | 21[b] | — | 0 | 21 |
| $qk^{k2}+/+tf^k \times T++tf/+qk^{kt1}t^{w5}+$ | 1 | — | 14 | 15 |
| $T++tf/+qk^{kt1}t^{w5}+ \times +qk^{k2}/tct^k+$ | 3 | 3 | 11 | 17 |
| $+qk^{k2}/tct^k+ \times qk^{kt1}T^{kt1}t^{w5}/+++$ | 1[e] | 23 | — | 33 |
| $+qk^{k2}/tct^k+ \times T++tf/+qk^{kt3}t^{w5}+$ | 0 | 1 | 15 | 16 |
| $Tqktf/+qk^{k2}+ \times T++tf/+qk^{kt3}t^{w5}+$ | 0 | 5[f] | 0 | 5 |
| $qk^{k2}+/+tf^k \times qk^{kt4}t^{w5}/++$ | 0 | — | 13 | 13 |

(B) Litters from crosses segregating for hemizygous $qk^k$ animals

| Parental genotype | Tail phenotype of offspring | | | |
|---|---|---|---|---|
| Female × Male | Short T | Tailless | Normal | Total |
| $tct^k+/+qk^k \times Tt^{Orl}/+$ | 0 | 5 | 19 | 24 |
| — | | | | |
| — | | | | |
| $+t^{w5}/qk^{k2}+ \times Tt^{Orl}/+$ | 0 | 25 | 66 | 91 |
| $Tt^{Orl}/+ \times qk^{kt1}t^{w5}/++$ | 0 | 0 | 14 | 14 |
| $+qk^{kt1}t^{w5}+/T++tf \times Tt^{Orl}/+$ | 15 | 0 | 20 | 35 |
| — | | | | |
| $qk^{kt3}t^{w5}/++ \times Tt^{Orl}/+$ | 4 | 0 | 3 | 7 |
| — | | | | |
| $qk^{kt4}t^{w5}/++ \times Tt^{Orl}/+$ | 8 | 0 | 16 | 24 |
| $Tt^{Orl}/+ \times qk^{kt4}t^{w5}/++$ | 0 | 0 | 12 | 12 |

As previously reported (Justice & Bode, 1986), crosses of $Tt^{Orl}/+$ with $Ttf/t^{w5}$ give viable, tailless animals (17/34 progeny). In addition, $qk^k$ and $qk^{k2}$ are fully viable when heterozygous with $t^{w5}$.

[a] No normal-tailed animals obtained from these crosses were quaking.

[b] All short-tailed progeny from these crosses were quaking.

[c] This normal-tailed animal was non-quaking and progeny testing showed it to be a recombinant of $++tf/+qk^k+$ genotype.

[d] The normal-tailed animal was non-quaking and progeny testing showed it to be a recombinant of $+qk^k+/++tf$ genotype. The lethal $qk^k$ allele could have been either $qk^k$ or $qk^{k2}$, we could not distinguish these possibilities by progeny testing.

[e] One short-tailed animal was born dead, so could not be progeny tested. A crossover between *tct* and *qk* could yield a ++ chromosome that would give a short-tailed animal in combination with $qk^{kt1}T^{kt1}t^{w5}$. The $qk^{kt1}T^{kt1}t^{w5}$ chromosome was generated from matings designed to determine the order of *T* and *qk* in *t*-haplotypes and the order of *T* and *qk* in the genetic formula reflects the results (Justice & Bode, in preparation).

[f] All tailless progeny from this cross were quaking, an observation which reflects the high transmission of $qk^{kt3}t^{w5}$-bearing sperm from the male.

(ii) *Hemizygotes of the three new* qk$^k$ *alleles exhibit developmental arrest at day 9 of embryogenesis*

The lethal gene associated with the $t^{w5}$-haplotype precludes a determination of the stage of embryonic death resulting from the $qk^{kt1}$ and $qk^{kt4}$ mutations in homozygotes. However, by using the $Tt^{Orl}$ deletion, it is possible to demonstrate both embryonic lethality and the time of developmental failure in hemizygous quaking embryos. $Tt^{Orl}/t^{w5}$ tailless hemizygotes are fully viable and fertile. The results of the embryological studies are shown in Table 2. Somite pair number of abnormal embryos ranged from 15–26. All had developed forelimb buds, but not hindlimb buds. Many (50%) of the defective embryos had varying degrees of haemorrhaging in the mesencephalon. Figure 1(*a*) is a photograph of two defective $qk^{kt1}$ $t^{w5}/Tt^{Orl}+$ embryos with a normal littermate. Figure 1(*b*) is a higher magnification of three defective $qk^{kt1}t^{w5}/Tt^{Orl}+$ embryos from the same litter, showing the haemorrhage. One embryo exhibits an enlarged pericardium. This abnormality was observed in eight of the abnormal embryos, but is probably not an effect of mutation at the *qk* locus. Seven abnormal embryos exhibited a cleavage through the head of the embryo, as if the head folds had not closed, but this was not a universal defect. The embryos shown in Fig. 1 are representative of all embryos from litters segregating for hemizygotes.

At 10·5–11 days, nine of the abnormal embryos were still alive (assessed by beating hearts), but at 12 days, all were dead. By 10·5 days and after, it was obvious that all abnormal embryos had stopped development at a common stage: somite pair number 15–26 or 9–9·5 days post coitum (p.c.).

Table 2. *Embryological observations of litters from matings segregating for* qk$^k$ *hemizygotes*

| Parental genotypes | | Embryos | | | | |
|---|---|---|---|---|---|---|
| Female × Male | Day | Normal | Abnormal[a] | Resorbed | Total | Abnormal or resorbed (%) |
| $qk^{k2}/+$ $Tt^{Orl}/+$ and $Tt^{Orl}/+$ $qk^{k2}+$ | 7–7·5 | 11 | 0 | 0 | 11 | 0 |
| | 8–8·5 | 10 | 0 | 0 | 10 | 0 |
| | 9–9·5 | 11 | 0 | 0 | 11 | 0 |
| | 10–10·5 | 18 | 11 | 1 | 30 | 40 |
| | 11–11·5 | 17 | 7 | 3 | 27 | 37 |
| | 12–12·5 | 13 | 3 | 3 | 19 | 31 |
| $qk^{kt1}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 7–7·5 | 13 | 0 | 0 | 13 | 0 |
| $qk^{kt1}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 8–8·5 | 11 | 0 | 0 | 11 | 0 |
| $Tt^{Orl}/+ \times qk^{kt1}$ $t^{w5}/+$ $+$ | 8–8·5 | 15 | 0 | 2 | 17 | 12 |
| $Tt^{Orl}/+ \times qk^{kt1}$ $t^{w5}/+$ $+$ | 9–9·5 | 9 | 0 | 0 | 9 | 0 |
| $qk^{kt1}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 10–10·5 | 10 | 2 | 0 | 12 | 17 |
| $qk^{kt1}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 11–11·5 | 17 | 5 | 0 | 22 | 23 |
| $Tt^{Orl}/+ \times qk^{kt1}$ $t^{w5}/+$ $+$ | 11–11·5 | 12 | 11 | 0 | 23 | 49 |
| $Tt^{Orl}/+ \times qk^{kt1}$ $t^{w5}/+$ $+$ | 15–15·5[b] | 2 | 0 | 3 | 5 | 60 |
| $Tt^{Orl}/+ \times qk^{kt4}$ $t^{w5}/+$ $+$ | 7–7·5 | 9 | 0 | 1 | 10 | 10 |
| $qk^{kt4}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 7–7·5 | 9 | 0 | 1 | 10 | 10 |
| $Tt^{Orl}/+ \times qk^{kt4}$ $t^{w5}/+$ $+$ | 11–1·5 | 10 | 16 | 0 | 26 | 61 |
| $qk^{kt4}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 12–12·5[b] | 24 | 5 | 1 | 30 | 20 |
| $qk^{kt4}$ $t^{w5}/+$ $+ \times Tt^{Orl}/+$ | 13–13·5[b] | 9 | 0 | 4 | 13 | 31 |
| $Tt^{Orl}+/+ + \times + t^{w5}/+$ $+$ | 13–15[c] | 40 | 0 | 1 | 41 | 3 |

Results from reciprocal crosses are combined in matings of $qk^{k2}/+$ with $Tt^{Orl}/+$. Reciprocal crosses are indicated in matings involving $qk^{kt1}$ $t^{w5}$and $qk^{kt4}$ $t^{w5}$. Heterozygous $qk^{kt1}$ $t^{w5}/+$ $+$ and $qk^{k4}$ $t^{w5}/+$ $+$ males will transmit the *t*-bearing sperm at a ratio of 90–100%, so we would expect nearly 50% abnormal embryos from matings with these males.

[a] The abnormal embryos had 15–26 somites and appeared normal macroscopically. The embryos were classified as abnormal because they had stopped development at this stage. Two embryos from matings segregating for $qk^{kt1}$ hemizygous embryos, three embryos from litters segegrating $qk^{k2}$ hemizygotes, and two embryos from litters segregating $qk^{kt4}$ hemizygotes had a cleavage through the head, appearing as though the head folds had not closed. Eight abnormal embryos had enlarged pericardiums. Twenty-eight, or about 50%, of the abnormal embryos from all three matings had haemorrhaging in the mesencephalon, cf. Fig. 1.

[b] Tail phenotype of normal embryos could be determined and was noted at these stages of development. All tails of normal embryos were consistent with that expected of heterozygotes or wild-type embryos, i.e. all were normal or short-tailed. No tailless were detected. Tail phenotype of abnormal embryos was difficult to determine because they were arrested in development, but in Fig. 1 the embryos exhibit some tail abnormalities which are consistent with the expected tailless phenotype.

[c] Tail phenotype was noted in these litters and included 21 tailless, 18 normal-tailed, and one short-tailed embryo, reflecting high transmission of $t^{w5}$-bearing sperm from the male.

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*1 (a)*

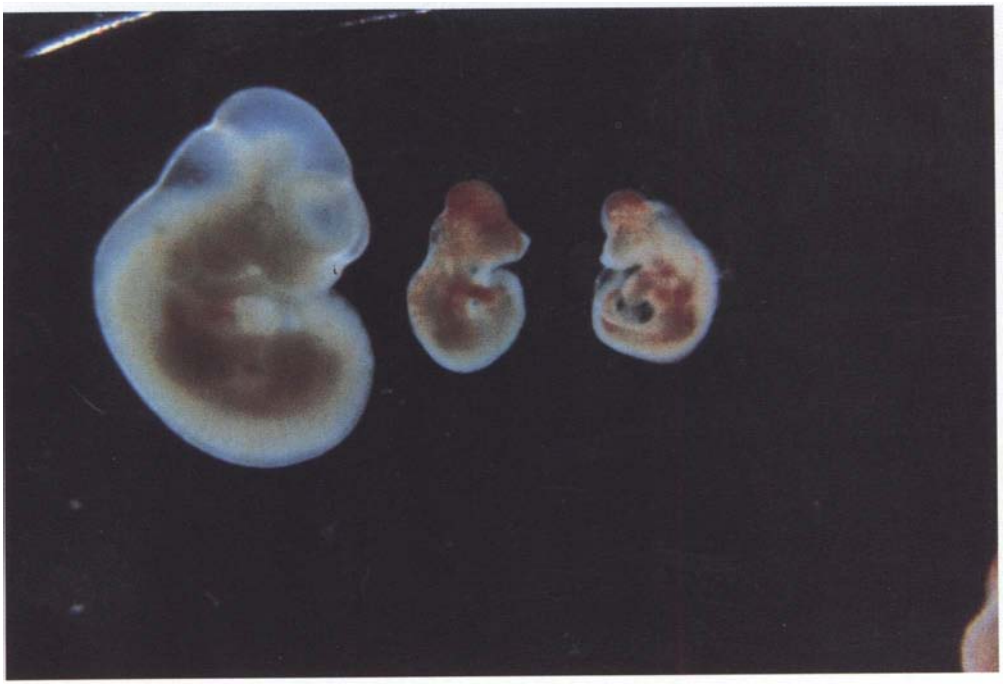

FIGURE 1(a): Two abnormal $Ti^{0rl}+/qk^{ktl}{}_{t}{}^{w5}$ embryos with a normal littermate. The parent female was 11 days past detection of the plug at the time of sacrifice.

*1 (b)*

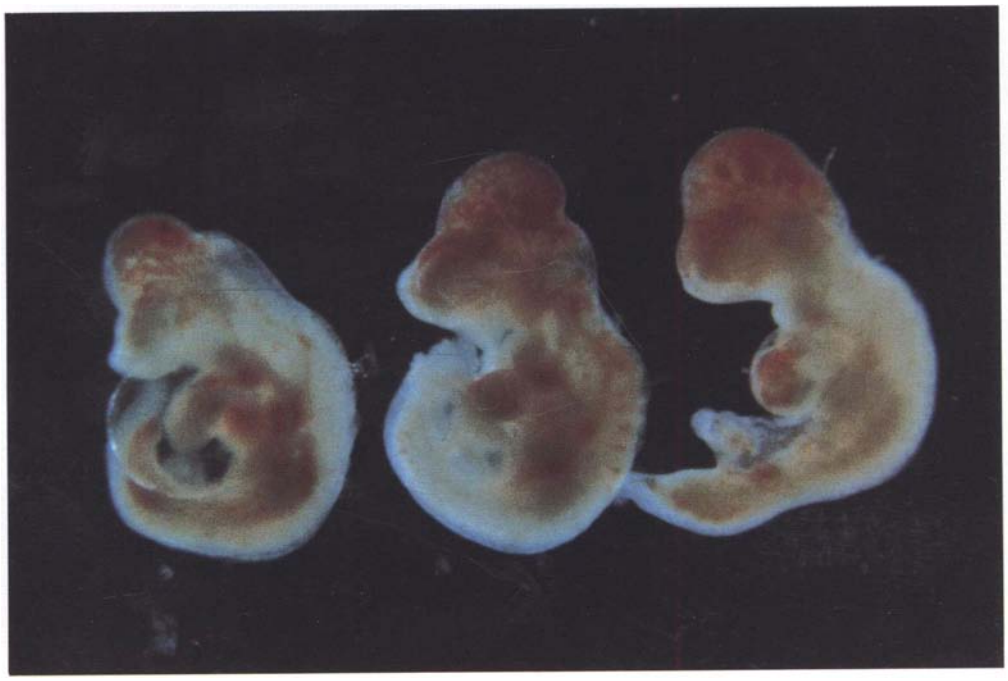

FIGURE 1(b): Three abnormal $Ti^{0rl}+/qk^{ktl}{}_{t}{}^{w5}$ embryos from the same litter as in Figure 1(a).



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(iii) *Homozygous* $qk^{k2}/qk^{k2}$ *embryos are abnormal at day 9*

Results of dissections from intercrosses of $qk^{k2}/+$ males and females are shown in Table 3. Some abnormal embryos detected as early as day 9 p.c. were very disorganized: all had abnormal diminished head folds, one had partial duplication of neural tissue and one had lost proper axis determination. The abnormal embryo shown in Fig. 2 had no detectable somites, diminished head folds and a large beating heart. One abnormal embryo had four abnormal small somite pairs, but had a very irregular neurocoel. With the exception of this one, all abnormal embryos were the size of 8–8·5 day embryos, but had no somites. In addition to these very defective abnormals, some embryos were arrested in development at 17–26 somite pairs or 9–9·5 days p.c. Another type of defective embryo was observed at a somewhat later stage of development, but occurred at a low frequency. These embryos had 37–43 somite pairs and all but one had slightly irregular neurocoels. All were dead at the time of observation.

(iv) *Compound heterozygotes of* $qk^{kt1}$ *and* $qk^{kt4}$ *with* $qk^{k2}$ *exhibit the same stages of developmental arrest*

Table 3 shows the results of matings of $qk^{k2}/+$ with $qk^{kt1}\ t^{w5}/++$ and $qk^{kt4}\ t^{w5}/++$. In litters segregating $qk^{kt4}/qk^{k2}$ compound heterozygotes, all abnormal embryos observed were arrested in development at 9–9·5 days p.c. with somite numbers ranging from 15–22. Three normal $qk^{k2}/qk^{kt1}$ embryos were disorganized, had no somites, and were the size of 8 day embryos, similar to $qk^{k2}$ homozygous embryos. Six abnormal $qk^{k2}/qk^{kt1}$ embryos were identical to those seen in matings of hemizygotes, i.e. arrested in development at the 18–26 somite stage. In addition, seven abnormal day 11 embryos were seen. These embryos had irregular neurocoels, as those seen in matings of $qk^{k2}$ heterozygotes. Thus, the characteristics of these abnormal embryos are identical to those of hemizygous and homozygous $qk^{k2}$ embryos.

Table 3. *Embryological observations of litters from matings segregating for* $qk^{k}$ *homozygotes and compound heterozygotes*

| Parental genotypes | | Embryos | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Abnormal[a] | | | | | % |
| Female × Male | Day | Normal | 8 day | 9 day | 11 day | Resorbed | Total | Abnormal or resorbed |
| $qk^{k2}/+ \times qk^{k2}+$ | 7–7·5 | 15 | 0 | 0 | 0 | 0 | 15 | 0 |
| | 8–8·5 | 13 | 2 | 0 | 0 | 0 | 15 | 13 |
| | 9–9·5 | 19 | 2 | 0 | 0 | 2 | 23 | 17 |
| | 10–10·5 | 14 | 2 | 1 | 0 | 0 | 17 | 18 |
| | 11–11·5 | 13 | 0 | 3 | 0 | 0 | 16 | 19 |
| | 12–12·5 | 30 | 2 | 1 | 0 | 2 | 35 | 14 |
| | 14–14·5 | 26 | 0 | 0 | 3 | 2 | 31 | 16 |
| | 15–15·5 | 12 | 0 | 0 | 2 | 4 | 18 | 33 |
| $qk^{k2}/+ \times qk^{kt1}\ t^{w5}/++$ | 7–7·5 | 16 | 0 | 0 | 0 | 1 | 17 | 6 |
| $qk^{kt1}\ t^{w5}/++ \times qk^{k2}/+$ | 12–12·5 | 50 | 3 | 6 | 2 | 0 | 61 | 18 |
| $qk^{k2}+ \times qk^{kt1}\ t^{w5}/++$ | 12–12·5 | 6 | 0 | 0 | 0 | 2 | 8 | 33 |
| $qk^{k2}/+ \times qk^{kt1}\ t^{w5}/++$ | 13–13·5 | 10 | 0 | 0 | 5 | 3 | 18 | 44 |
| $qk^{kt1}\ t^{w5}/++ \times qk^{k2}/+$ | 15–15·5 | 11 | 0 | 0 | 0 | 4 | 15 | 27 |
| $qk^{kt4}\ t^{w5}/++ \times qk^{k2}/+$ | 7–7·5 | 24 | 0 | 0 | 0 | 0 | 24 | 0 |
| | 9–9·5 | 2 | 0 | 0 | 0 | 1 | 3 | 33 |
| | 10–10·5 | 9 | 0 | 2 | 0 | 0 | 11 | 18 |
| | 11–11·5 | 40 | 0 | 9 | 0 | 1 | 50 | 20 |
| | 13–13·5 | 6 | 0 | 0 | 0 | 2 | 8 | 25 |
| $+/+ \times qk^{k2}/+$ | 13–14·5 | 36 | 0 | 0 | 0 | 4 | 40 | 10 |
| $qk^{k2}/+ \times +/+$ | 12·5–13·5 | 45 | 0 | 0 | 0 | 2 | 47 | 4 |

Heterozygous $qk^{kt1}\ t^{w5}/++$ and $qk^{kt4}\ t^{w5}/++$ males will transmit the *t*-bearing sperm with distortion, so one would expect almost 50% abnormal embryos from matings with $qk^{kt}\ t^{w5}$ males.

[a] The abnormal embryos were of several types. In the first two matings, abnormal embryos of approximately 8 day size were very disorganized, all with abnormal diminished head folds and only one with detectable somites. One had partial duplication of neural tissue and one had loss of axis determination. In addition, some abnormal embryos from these crosses had 17–26 somites but were arrested in development as compared with normal littermates and are designated day 9. A few abnormal embryos of 37–43 somites that had irregular neurocoels occurred at a frequency of about 10% in late dissections when they could be detected. In $qk^{k2}/+ \times qk^{kt4}\ t^{w5}/++$ matings, all abnormal embryos were similar to those of $qk^{k}$ hemizygotes, i.e. arrested in development at the 15–22 somite pair stage.

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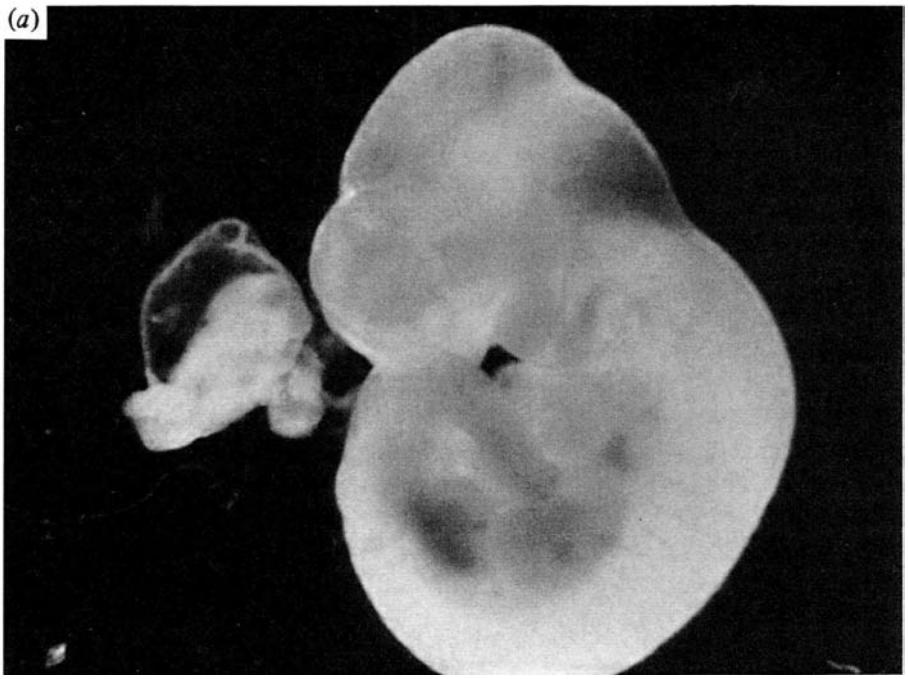


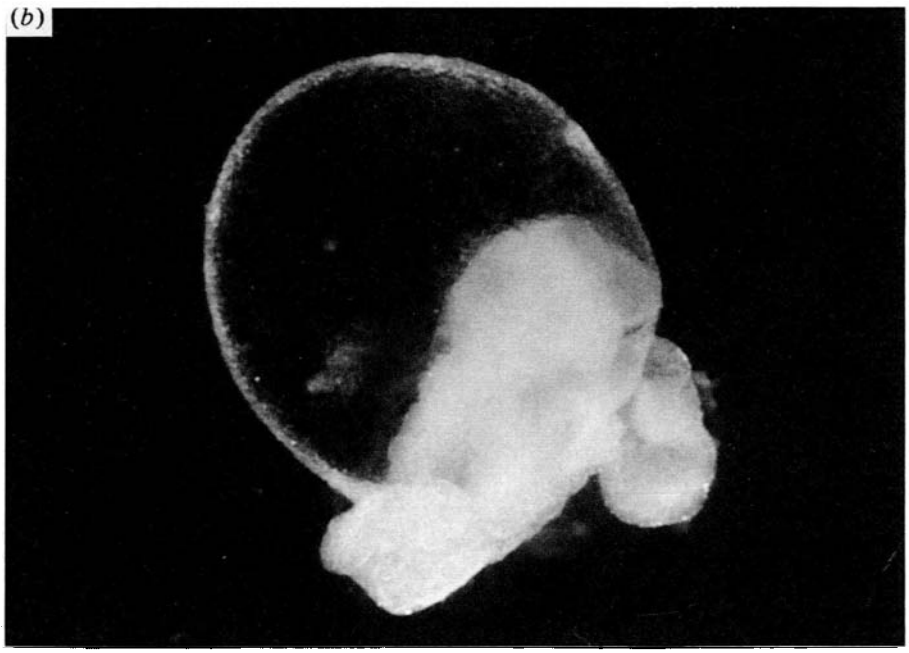


Fig. 2(*a*) An abnormal $qk^{k2}$ homozygous embryo from a mating of $qk^{k2}$ heterozygotes with a normal littermate dissected later on day 9·5 past detection of the vaginal plug. (*b*) The same abnormal $qk^{k2}$ homozygous embryo as in Fig. 2(*a*), but shown at a higher magnification. The protrusion at the right is a large beating heart. The embryo was very disorganized with abnormal reduced head folds, no macroscopically detectable somites, and a large unidentified sac.

## 4. Discussion

Evidence is presented here to confirm that the new $qk^{k}$ alleles induced by ethylnitrosourea mutagenesis are recessive lethal mutations that cause the embryonic death of homozygotes. The homozygous lethality of $qk^{kt1}$ and of $qk^{kt4}$ was not determined directly, but can be inferred from the failure to complement $qk^{k2}$ and the deficiency $Tt^{Orl}$. A fourth allele of *qk*, *l(17)-1* $^{Wis}$, exhibits a transient and less severe quaking phenotype when heterozygous with *qk*. Animals exhibit a tremor from about two weeks to about five weeks of age and then are indistinguishable from normal (Shedlovsky & Dove, unpublished results). In contrast to the $qk^{k}$ alleles, this allele was isolated in a three generation ENU-mutagenesis experiment designed to obtain lethal mutations linked to *tf* on chromosome 17. It fails to complement any of the three $qk^{k}$ alleles

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(Shedlovsky, Guenet, Dove *et al.* in the press). Moreover, homozygotes die at about 8 days of gestation (Shedlovsky *et al.* 1986). These observations substantiate our evidence that mutation by ENU at the quaking locus can cause the embryonic death of homozygotes.

$Tt^{orl}/qk$ males are sterile (Silver, Lukralle & Garrels, 1983). In contrast, males of $qk^{k}/qk$ and $l(17)\text{-}1^{Wis}/qk$ genotype are fertile. Therefore, male sterility is probably not a result of simple mutation at the *qk* locus. It is striking that the spontaneous mutation causing the original *qk* resulted in viable quaking and male sterile phenotypes, whereas four ENU-induced alleles, presumably point mutations, resulted in lethal but male fertile phenotypes. Accordingly, the male sterility of the *qk* mutation that previously defined the locus may be due to some complexity of the mutational change that resulted in that allele.

In three examples, $qk^{k2}$, $qk^{ktl}$ and $qk^{kt4}$, the mutation fails to complement the quaking phenotype of *qk*. These mutations were selected on the basis of their quaking phenotype and were determined to be embryonic lethal alleles because of the experiments reported here. In another example, $l(17)\text{-}1^{Wis}$, the mutation shows only partial complementation of this phenotype. This mutation was selected on the basis of its homozygous embryonic lethality. Consequently, it is probable that mutation in the same essential gene has caused both the quaking phenotype and embryonic death. An alternative explanation of the effects of the various alleles of the *qk* locus might be that myelination, embryonic development, and spermatid differentiation are controlled by three separate but closely linked genes, and that the $qk^{k}$ and $l(17)\text{-}1^{Wis}$ alleles affect two of these loci. It is notable, however, that of the mutations induced by ENU at the loci screened by the viable alleles in the specific-locus test of Russell (1951), with the exception of the *d* (dilute) and *s* (piebald) loci, the proportion that are homozygous lethal is very low, less than 5%. Furthermore, 0/267 independent mutations isolated during these ENU-mutagenesis experiments at the closely linked *d* and *se* (short-ear) loci have an effect on both loci. When the conditions of a radiation experiment are known to give deletions, mutations affecting both loci are not uncommon (Russell, 1982). Therefore, ENU is more likely to induce gene mutations that reflect the properties of a single locus, rather than the complexities of the mutational change. Moreover, it is highly improbable that four independently induced mutations at this locus would have affected two loci separately affecting myelination and the same function required for embryonic development. We conclude that the wild type quaking gene product is necessary both for normal myelination and for embryonic survival.

The abnormal embryos examined in this report do not show a single precise point of embryonic death, but all die at a restricted period during mid-gestation. All hemizygous embryos are essentially normal, but arrested in development. Some embryos from *inter-se* matings of $qk^{k2}$ heterozygotes are macroscopically abnormal at 9 days of development. This difference may be a result of modifying genes present in the background, or may be an indication of an allelic distinction. However, we would expect the haploinsufficiencies associated with the $Tt^{Orl}$ deficiency to cause the more severe defect.

In crosses segregating for $qk^{k2}$ homozygotes and crosses segregating for $qk^{k2}/qk^{ktl}$ compound heterozygotes, we see an additional lethal phenotype: some embryos die at the 37–43 somite stage, or approximately 11 days p.c. This type of abnormal embryo was not found in controls, but may be a background lethal. Alternatively, we could postulate that these are also defective $qk^{k2}/qk^{k2}$ embryos that have survived to a later stage of development. The lethal phenotype observed is not precise, and these embryos may be a late extreme of abnormal. Because the mutation induced by ENU may be a single base change, the lethal phenotype in homozygotes may be somewhat 'leaky', i.e. the gene product may have some residual normal function. Until we can identify homozygous $qk^{k}$ embryos by molecular or chromosomal markers, this observation will remain unresolved.

These alleles should be useful in experiments to determine the function of the *qk* gene in embryonic development and in myelination. Some authors have speculated that the *qk* gene product has a role in the biogenesis of very long chain fatty acids (Baumann *et al.* 1972; Bourre *et al.* 1986). Experiments are in progress to determine the lipid and fatty acid content of defective embryos in comparison with normal embryos of the same stage. If very long chain fatty acids are detectable at this stage of embryogenesis, we may be able to show a difference. In addition, we are characterizing the lipid and fatty acid composition in brain myelin of each of the $qk^{k}$ alleles compound with *qk*. Preliminary results suggest that $qk^{ktl}$ has a dominant effect on the lipid composition of brain myelin; furthermore, some $qk^{ktl}/+$ heterozygotes have seizures and a mild quaking phenotype in old age (Justice and Bode, unpublished results). Thus, $qk^{ktl}$ has a semidominant effect.

The abnormal development of myelin in *qk/qk* homozygotes does not give a full picture of the function of the quaking gene product. The isolation of these new mutations demonstrates the importance of obtaining additional alleles of known genes to give a complete representation of gene function. In addition, the collection of different alleles, including the $Tt^{Orl}$ deletion, and the availability of probes for this region of chromosome 17 (Fox *et al.* 1985; Mann, Silver & Elliott, 1986) should prove useful in the molecular cloning and analysis of the *qk* locus.

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## References

Baumann, N. A., Bourre, J. M., Jacque, C & Pollett, S. (1972). Genetic disorders of myelination. In *Lipids, Malnutrition and the Developing Brain* (ed. K. Elliott and J. Knight). Elsevier, Amsterdam.

Bennett, W., Gall, A. M., Southard, J. L. & Sidman, R. L. (1971). Abnormal spermiogenesis in quaking, a myelin deficient mutant mouse. *Biology of Reproduction* **5**, 30–58.

Bode, V. C. (1984). Ethylnitrosourea mutagenesis and the isolation of mutant alleles for specific genes located in the *t* region of mouse chromosome 17. *Genetics* **108**, 457–470.

Bourre, J.-M. Boiron, F., Cassagne, C., Dumont, O., Leterrier, F., Metzger, H. & Viret, J. (1986). Biochemical and physicochemical determinations in a premyelin fraction obtained by zonal centrifugation in normal mouse and in dysmyelinating mutants (quaking, shiverer, and myelin-deficient). *Neurochemical Pathology* **4**, 29–42.

Fox, H. S., Martin, G. R., Lyon, M. F., Hermann, B., Frischauf, A.-M., Lerarch, H. & Silver, L. M. (1985). Molecular probes define different regions of the mouse *t*-complex. *Cell* **40**, 63–69.

Justice, M. J. & Bode, V. C. (1986). Induction of new mutations in a mouse *t*-haplotype using ethylnitrosourea mutagenesis. *Genetical Research* **47**, 187–192.

Mann, E. A., Silver, L. M. & Elliott, R. W. (1986). Genetic analysis of a mouse *t* complex locus that is homologous to a kidney cDNA clone. *Genetics* **114**, 993–1006.

Popp, R. A., Bailiff, E. G., Skow, L. C., Johnson, F. M. & Lewis, S. E. (1983). Analysis of a mouse $\alpha$-globin gene mutation induced by ethylnitrosourea. *Genetics* **105**, 157–167.

Peters, J., Andrews, S. J., Loutit, J. F. & Glegg, J. B. (1985). A mouse $\beta$-globin mutant that is an exact model of hemoglobin Rainier in man. *Genetics* **110**, 709–721.

Russell, W. L. (1951). X-ray-induced mutations in mice. *Cold Spring Harbor Symposia on Quantitative Biology* **16**, 327–335.

Russell, W. L. (1982). Factors affecting mutagenicity of ethylnitrosourea in the mouse specific-locus test and their bearing on risk estimation. In *Environmental Mutagens and Carcinogens* (ed. T. Sugimura, S. Kondo and H. Takebe), pp. 59–70. New York: Alan R. Liss.

Russell, W. L., Kelly, E. M., Hunsicker, P. R., Bangam, J. W., Maddux, C. & Phipps, E. L. (1979). Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. *Proceedings of the National Academy of Sciences, U.S.A.* **76**, 5818–5819.

Samorajski, T., Friede, R. L. & Reimer, P. R. (1970). Hypomyelination in the quaking mouse. A model for the analysis of disturbed myelin formation. *Journal of Neuropathology and Experimental Neurology* **29**, 507–523.

Shedlovsky, A., Guenet, J.-L., Johnson, L. L. & Dove, William, F. (1986). Induction of recessive lethal mutations in the *T/t-H-2* region of the mouse genome by a point mutagen. *Genetical Research* **47**, 135–142.

Sidman, R. L., Dickie, M. M. & Appel, S. H. (1964). Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. *Science* **144**, 309–311.

Silver, L. M., Lukralle, D. & Garrels, J. I. (1983). $T^{Orl}$ is a novel, variant form of mouse chromosome 17 with a deletion in a partial *t*-haplotype. *Nature* **301**, 422–424.

Theiler, K. (1972). *The House Mouse*. New York: Springer-Verlag.

Vogel, E. & Natarjan, A. T. (1979). The relation between reaction kinetics and mutagenic action of mono-functional alkylating agents in higher eukaryotic systems. 1. Recessive lethal mutations and translocations in *Drosophila*. *Mutation Research* **62**, 51–100.