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Small eyes (*Sey*): a homozygous lethal mutation on chromosome 2 which affects the differentiation of both lens and nasal placodes in the mouse

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SUMMARY

Small eyes (*Sey*) is a semidominant, homozygous lethal mutation in the mouse (Roberts, 1967). It is allelic with Sey^H, a radiation-induced homozygous prenatal lethal which has been mapped on chromosome 2. The effect of the *Sey* mutation is apparently limited to the growth and differentiation of the presumptive lens and nasal placodes. Homozygous *Sey*/*Sey* embryos can be distinguished as early as 10·5 days *post coitum* (*p.c.*); the optic vesicles grow out, but the ectoderm does not give rise to a lens and nasal pits never form. Immunohistochemical studies show that the distribution of the extracellular matrix glycoprotein laminin is not significantly different in the cephalic region of *Sey*/*Sey* versus *Sey*/+ or +/+ embryos. *Sey*/*Sey* embryos develop to term but without eyes or nose, and die soon after birth. Further analysis of *Sey*/*Sey* embryos may throw light on the mechanisms underlying morphogenesis of craniofacial structures in mammals.

INTRODUCTION

In the mammalian embryo, the development of the head and face involves the co-ordinated growth and interaction of many different tissues. These include the brain and optic vesicles, the mesenchymal cells derived from the cephalic neural crest, and the ectoderm of the maxillary and mandibular processes. In addition, the olfactory epithelium, lens, sensory ganglion V and otic vesicles are derived from paired cephalic placodes on either side of the neural plate (Slavkin, 1979; Nieuwkoop, Johnen & Albers, 1985; Le Douarin, 1982; Zimmerman, 1984). Because of the relative inaccessibility of the mammalian embryo, most of the

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experimental work on the lineage and morphogenesis of craniofacial structures, including the cephalic placodes, has been done with avian embryos (e.g. Yee & Abbott, 1978; Tamarin, Crawley, Lee & Tickle, 1984; Couly & Le Douarin, 1985). However, the mouse offers the advantages of a genetic approach, coupled with the increasing availability of cloned genes as probes for the precise mapping and isolation of mutant loci and deletions. Several known mouse mutants develop craniofacial malformations as a consequence of primary defects in processes such as neural crest migration or the formation of cartilage, e.g. *patch* (*Ph*), and *cartilage matrix deficiency* (*cmd*) (Green, 1981; Brown, 1980). However, other mutations appear to have more specific effects on the growth and differentiation of facial primordia and may throw light on the nature of tissue interactions during development. For example, mice homozygous for the recessive mutation *eyeless*-1 (*ey*-1/*ey*-1) are anophthalmic because of a failure in lens induction by the optic cup (Chase & Chase, 1941; Silver & Hughes, 1974; Harch, Chase & Gonsalves, 1978; Webster, Silver & Gonsalves, 1984). In this study we show that the effect of the dominant homozygous lethal mutation, *Small eyes* (*Sey*) (Roberts, 1967) is apparently limited to the development of the presumptive lens and nasal placodes; homozygous *Sey*/*Sey* embryos have no eyes or nose and die soon after birth. We present evidence that *Sey* is allelic with a radiation-induced mutation, Sey^{H}, a homozygous prenatal lethal mutation which has been mapped on chromosome 2.

It had been suggested that the abnormalities of eyes of heterozygous *Sey*/+ mice were associated with the accumulation around the lens of excess extracellular matrix glycoprotein containing more sialic acid than normal (Pritchard, Clayton & Cunningham, 1974). However, in the course of this study we found no difference in the distribution of the extracellular matrix glycoprotein, laminin, in the cephalic region of mutant and normal embryos.

MATERIALS AND METHODS

Mice

Two *Sey*/+ males were kindly provided by Dr Ruth Clayton, Department of Genetics, Edinburgh. They were mated with female C57BL/10Nimr mice at the National Institute for Medical Research, London. Heterozygous female offspring have now been back-crossed to B10 males for more than four generations. For the experiments described here timed embryos were obtained by mating heterozygous males and females of the first and second backcross generations (see Table 1). Noon of the day on which the plug is observed is assumed to be 0·5 days *p.c.*

The Sey^{H} mutation was first identified by Rita Phillips in the offspring of an X-irradiated female (Lyon, Phillips & Fisher, 1979). It has been maintained in the MRC Radiobiology Unit, Harwell, on a random bred background.

Histology

Embryos were fixed in 2 % glutaraldehyde in phosphate-buffered saline (PBS), embedded in LKB Historesin and horizontal sections cut on glass knives at 5 μm. Neonatal animals were fixed

in formol saline, the heads embedded in paraffin wax, and coronal sections cut at 6 μm. A one in ten series was stained with thionin.

Immunohistochemistry

Embryos were fixed in 2% paraformaldehyde, 0·075 M-lysine, 0·01 M-sodium periodate in 0·0375 M-sodium phosphate pH 6·2 overnight at 4°C (McLean & Nakane, 1974). They were transferred to 1 M-sucrose in PBS, left overnight at 4°C, embedded in OCT embedding medium and cryostat sections cut at 7–10μm. Sections were incubated overnight at 4°C with a 1:200 dilution of affinity-purified rabbit anti-mouse laminin serum (kindly provided by Dr Janet Winter, Sandoz Institute, London). After washing, the sections were incubated for 2 h at room temperature with 1:100 biotinylated donkey anti-rabbit immunoglobulin followed by further washing and incubation for 2 h at room temperature with 1:100 fluorescein–Streptavidin (Amersham International).

RESULTS

Identification of homozygous Sey/Sey *embryos*

Embryos from *Sey*/+ × *Sey*/+ crosses were examined between 9·5 and 18·5 days *p.c.* under a ×40 stereomicroscope for differences in external morphology including overall size of the extraembryonic membranes, placenta and foetus, number of digits, tail length, and craniofacial development. Mutant embryos could be unambiguously identified after 10·5 days *p.c.* and then only by differences in the morphology of the developing eye and nose. At 10·5 days *p.c.* one class of embryo had nasal pits and optic vesicles with lenses; the other class had no nasal pits and the optic vesicles, which appeared to be of normal size, nevertheless did not contain a lens (Fig. 1A,B and Table 1). Other cranial features, such as maxillary and mandibular processes, hyoid arch and otic vesicles, appeared identical between the two groups (Fig. 1C). After 11·5 days *p.c.*, the embryos that had lenses were also clearly distinguished by the presence of pigment in the eye, while the empty optic vesicles of the other class never showed pigment. The embryos with pigmented eyes had well-developed nasal tissue, visible externally as lateral and medial nasal processes and nasal cavities. In contrast, the embryos with abnormal eyes only developed two small frontonasal protuberances which in due course fused with the maxillary processes (see also Fig. 4B).

From the number of embryos in each class (Table 1) we conclude that those with abnormal eyes and noses are homozygous *Small eyes* (*Sey*/*Sey*) (101/367 = 27·5%). At 18·5 days *p.c.* these embryos were clearly viable, and it seemed likely that they would develop to term. However, pups with the expected phenotype were not normally found. Therefore, in one experiment we separated newborn young from the mother immediately after birth; in a litter of 11 pups, 2 had neither eyes nor nasal tissue, including nostrils. In all other respects, they appeared normal externally and the maxillae had a normal complement of whisker follicles. Histological examination confirmed the absence of eyes and nasal structures and also revealed the absence of olfactory bulbs. In contrast, the glandular tissue of the adenohypophysis and the intermediate pituitary both appeared normal (data not shown).

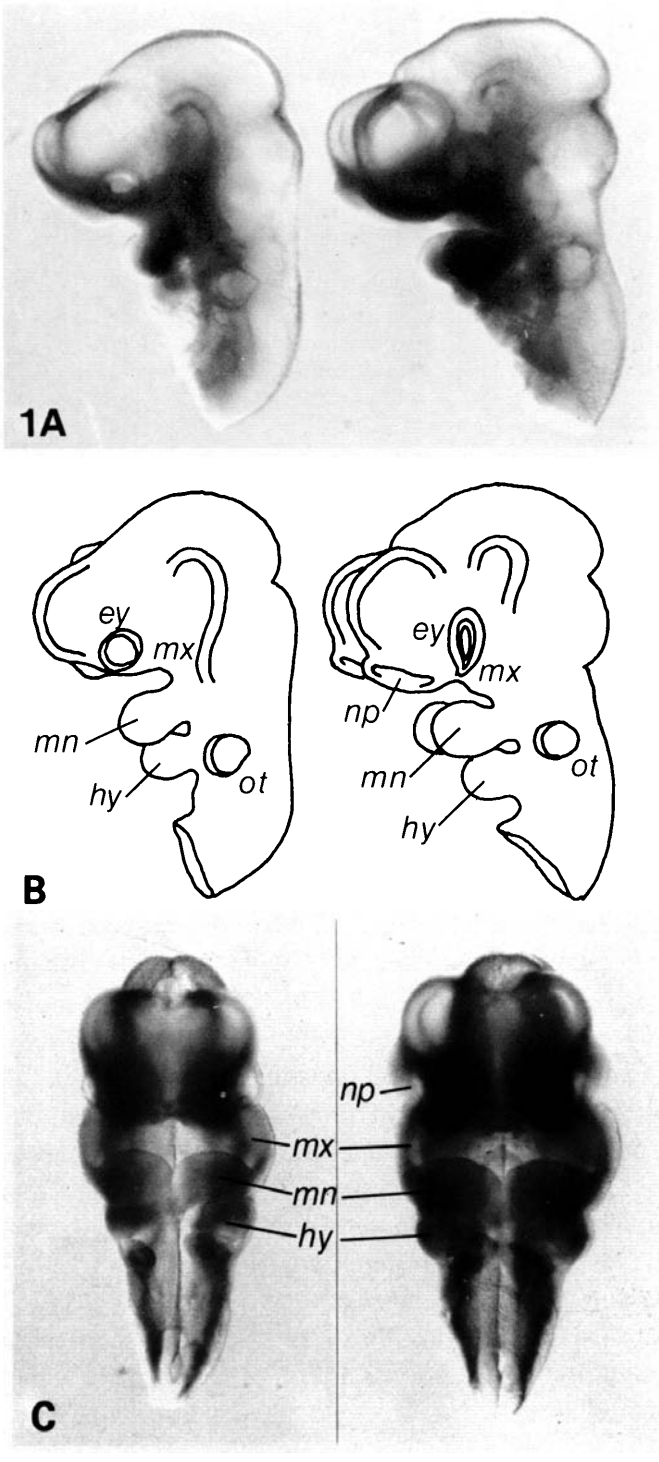


Fig. 1. External morphology of *Sey/Sey* embryo and littermate at 10·5 days *p.c.*

(A) Photomicrograph of littermates from a cross (*Sey*/+ × *Sey*/+). A homozygous mutant is on the left and a normal or heterozygous embryo on the right. For clarity only the anterior part of the embryo is shown; no significant difference was seen in the parts not shown.

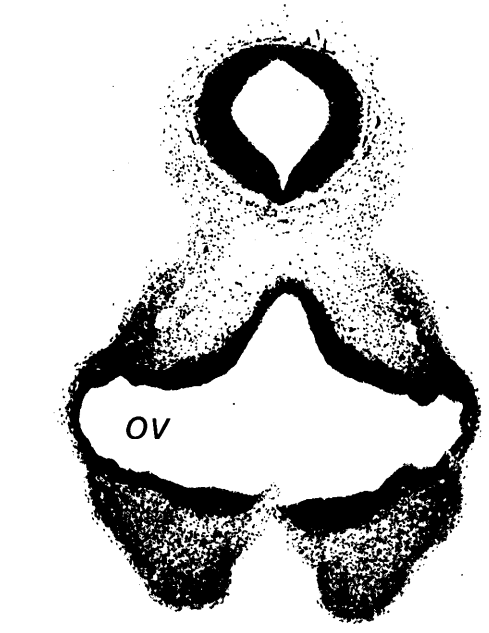
(B) Line drawing of A showing eye (*ey*), nasal pit (*np*), maxillary process (*mx*), mandibular process (*mn*), hyoid arch (*hy*) and otic vesicle (*ot*).

(C) Photomicrograph of same embryos as in A, but viewed from the ventral surface.

Table 1. *Phenotype of embryos resulting from crosses between heterozygous* Small eyes (Sey/+ × Sey/+)*

Age of embryos (days *p.c.*)	Number of litters	Total number of implantation sites examined	Small implantation sites (moles) or very retarded embryos	Embryos without lens and nasal pits or eyes and nose†	Embryos with both lens and nasal pits or eyes and nose
10·5	14	133	11	32	90
11·5	8	71	4	17	50
12·5	3	30	1	10	19
13·5	6	52	3	14	35
14·5	2	18	3	3	12
15·5	2	18	0	10	8
16·5	2	7	0	1	6
17·5	1	9	0	4	5
18·5	3	29	0	10	19
Total		367	22	101	244

* No significant difference was observed in the distribution of embryonic phenotypes depending on the backcross generation of the parents.
† After 11·5 days *p.c.*, embryos in this class also had no pigment in the eye region. At 10·5 days *p.c.* optic vesicles were present but they did not contain a lens (see Figs 1–3).



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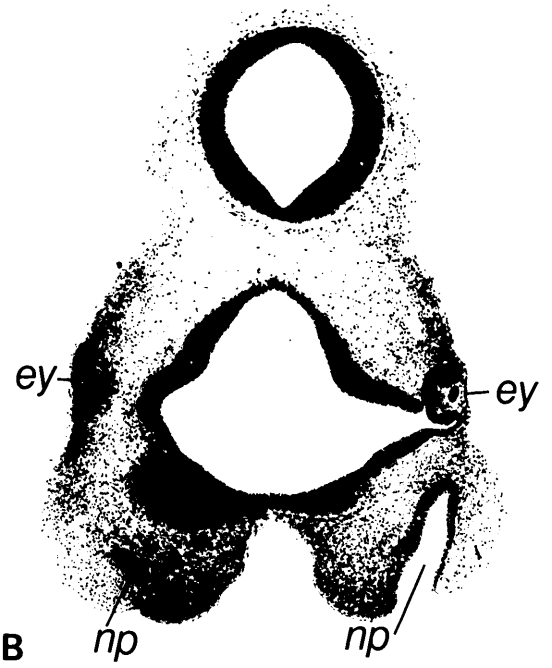

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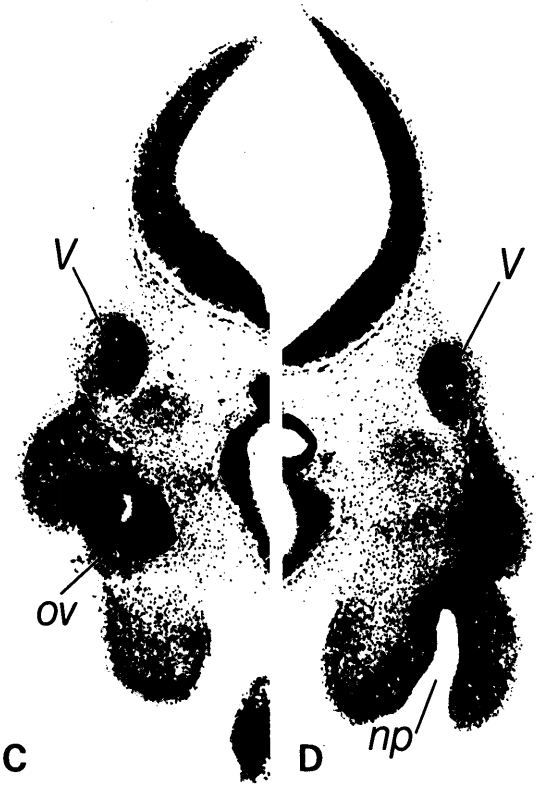

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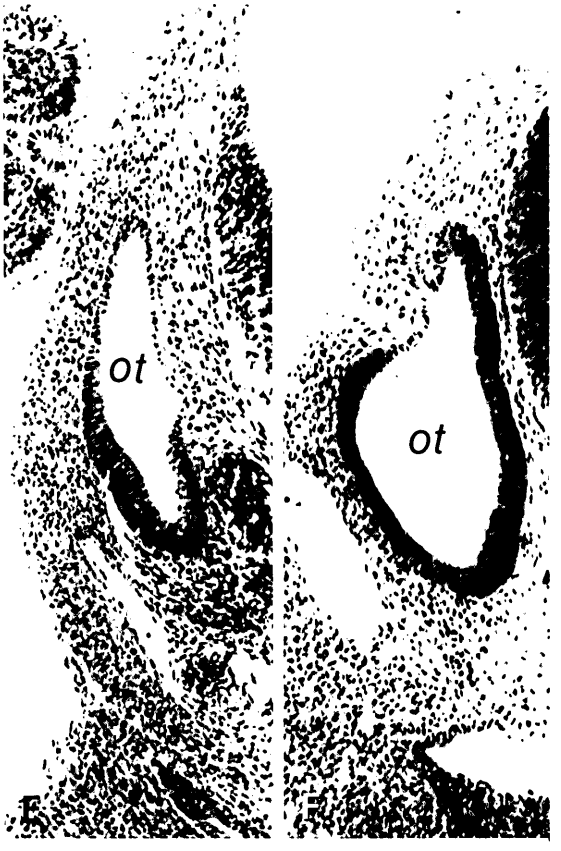

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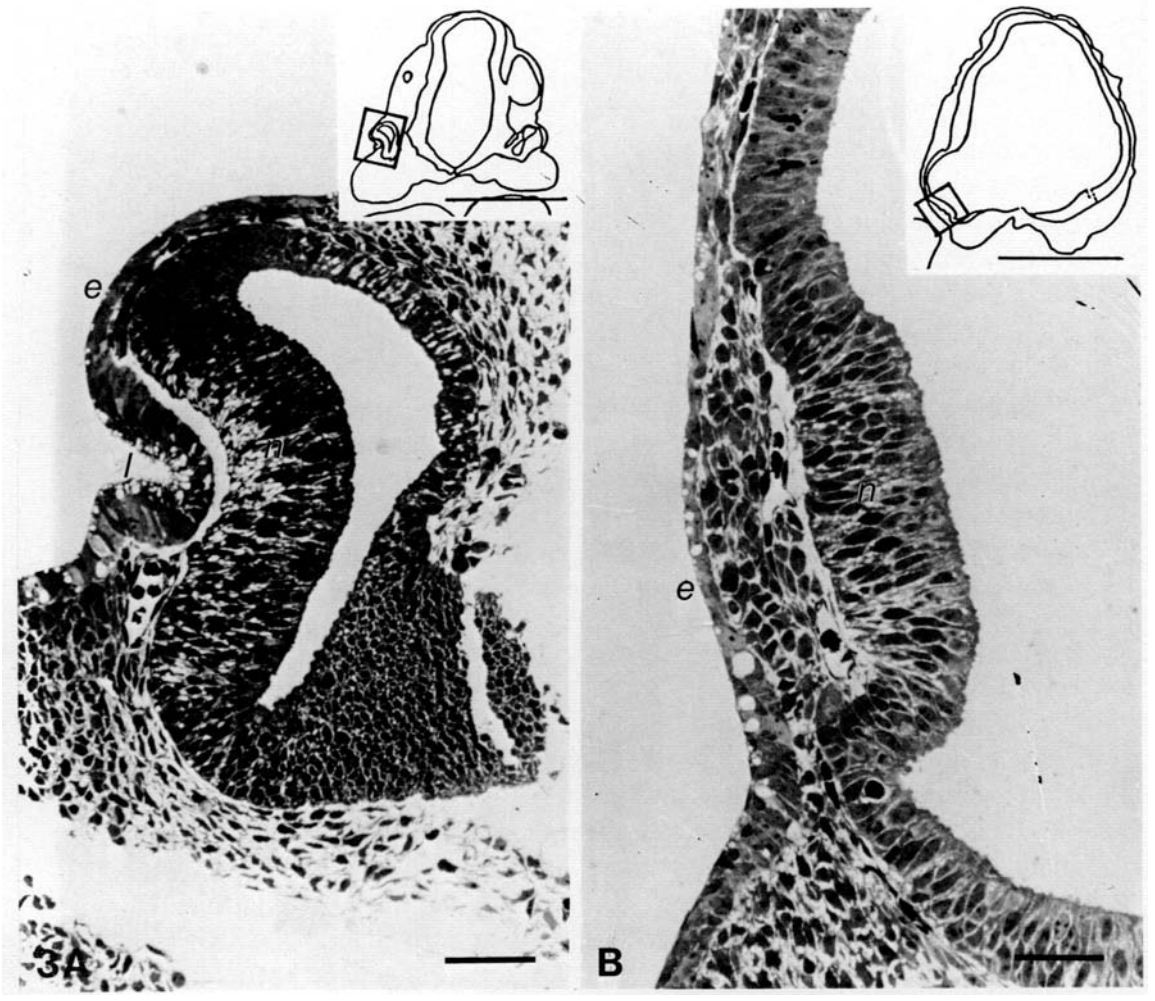


Fig. 3. Relationship between optic vesicle and presumptive lens ectoderm in normal and mutant embryos at 10·5 days *p.c.*

(A) Normal lens induction in coronal section of 10·5 days *p.c.* *Sey*/+ or +/+ embryo. Scale bar, 50 μm. Inset, low power tracing; region photographed is boxed. Scale bar, 1 mm.

(B) Homozygous mutant (*Sey*/*Sey*) littermate of A. The optic vesicle and presumptive lens ectoderm are separated by mesoderm. Scale bar, 20 μm. Inset, low power tracing; region photographed is boxed. Scale bar, 1 mm. n, neuroectoderm; e, head ectoderm; l, lens pit.

Fig. 2. Internal morphology of *Sey*/*Sey* embryo and littermate at 10·5 days *p.c.* Panels A–F show horizontal sections at different regions on one *Sey*/*Sey* embryo (A,C,E) and one *Sey*/+ or +/+ littermate (B,D,F). Both embryos were 10·5 days *p.c.*, but the homozygous mutant was in this case somewhat smaller than its littermate.

(A) *Sey*/*Sey* embryo showing optic vesicles (*ov*) but absence of both eye cup, lens and nasal pits.

(B) Littermate, showing eye cups with lens (*ey*) (glancing section on left), and nasal pit (*np*).

(C) Half of *Sey*/*Sey* embryo, showing sensory ganglion V (*V*) and edge of optic vesicle (*ov*).

(D) Half of littermate, at about the same level as C.

(E) *Sey*/*Sey* embryo showing otic vesicle (*ot*).

(F) Littermate showing otic vesicle (*ot*).

Scale bar, 0·25 cm.

Morphology of the eye of heterozygous Sey/+ *embryos*

Heterozygous *Sey*/+ and wild-type +/+ embryos could not be distinguished externally. However, histological examination of serial sections of the eyes of 13·5 and 15·5 days *p.c.* litter mates revealed several differences. The major distinguishing features of *Sey*/+ eyes were the small size of the lens, which contained many vacuolated cells, and the folding of the retinal epithelium around the anterior of the lens. In some cases, the small lens was also incompletely separated from the overlying ectoderm.

Internal morphology of the eye and nose region of 10·5 days p.c. *homozygous (*Sey/Sey*) embryos*

Homozygous *Sey*/*Sey* embryos at 10·5 days *p.c.* were serially sectioned at 5 μm intervals and compared with *Sey*/+ or +/+ litter mates. As shown in Fig. 2A, the homozygous mutant embryos had well-developed optic vesicles, but no lens placodes. In all embryos examined, the optic epithelium did not appear to come into direct contact with the overlying ectoderm and was separated from it by a layer of mesodermal cells (Fig. 3A,B). Nasal placodes or pits were also absent. However, both the cephalic ganglia V and the otic vesicles appeared normal (Fig. 2C–F).

Fig. 4 shows the distribution of laminin in a homozygous mutant compared with a normal or heterozygous 10·5 days *p.c.* embryo. There was no significant difference between the two classes in the thickness and continuity of the basal laminae underlying the neuroepithelium and the ectoderm, either in the region of the optic vesicles or the nasal pits (cf. also the pattern of laminin distribution observed in the developing eye of rat embryos by Parmigiani & McAvoy (1984)). Moreover, in all the homozygous mutant embryos examined there was no abnormal accumulation of laminin in any other part of the head region.

Mapping the Sey *mutation*

The *Sey* mutation has many similarities with a radiation-induced, dominant, homozygous prenatal lethal mutation, now known as *Sey*-Harwell (Sey^{H}), which has been maintained in the MRC Radiobiology Unit, Harwell, UK, since 1975. By means of the cross *Sey*/+ × Sey^{H}/+ the two mutations were shown to be allelic (Table 2); of 69 informative embryos, 17 (25 %) lacked both eyes and nose. At 13·5 days *p.c.* the double heterozygous mutant embryos were somewhat smaller and more retarded in facial development than either their littermates (Fig. 5) or *Sey*/*Sey* embryos. The maxillae had not fused and there was only a small bilobed protrusion in the frontonasal position. In some 13·5 days *p.c.* embryos this mass was fused with a maxillary process on one side. A few small blebs were present in the area normally occupied by the eye but histological examination showed that these were superficial, and did not involve the brain.

Heterozygous Sey^{H}/+ mice differ from *Sey*/+ in various ways. They are usually small at birth, and the eyes as well as being small often have coloboma which is

visible in embryos from around 14·5 days gestation. At weaning age of 2·5 weeks $Sey^H/+$ are still typically somewhat smaller than their normal sibs and usually have a white spot or streak on the belly. Sometimes the pinnae are also affected, with one or both ears small and low set. Both sexes are fertile, but females are poor breeders. There is a marked deficiency of $Sey^H/+$ animals at birth, suggesting prenatal death, of which the cause is not known. Homozygous Sey^H/Sey^H embryos die prenatally, but the poor breeding performance of $Sey^H/+$ females has so far prevented detailed study. Among the few data obtained, there have been no embryos resembling *Sey/Sey*, but instead marked death of early embryos, both before and after implantation. However, some of the dead embryos may be $Sey^H/+$ (in view of the deficiency of these at birth) and hence the exact time of death of Sey^H/Sey^H is unknown.

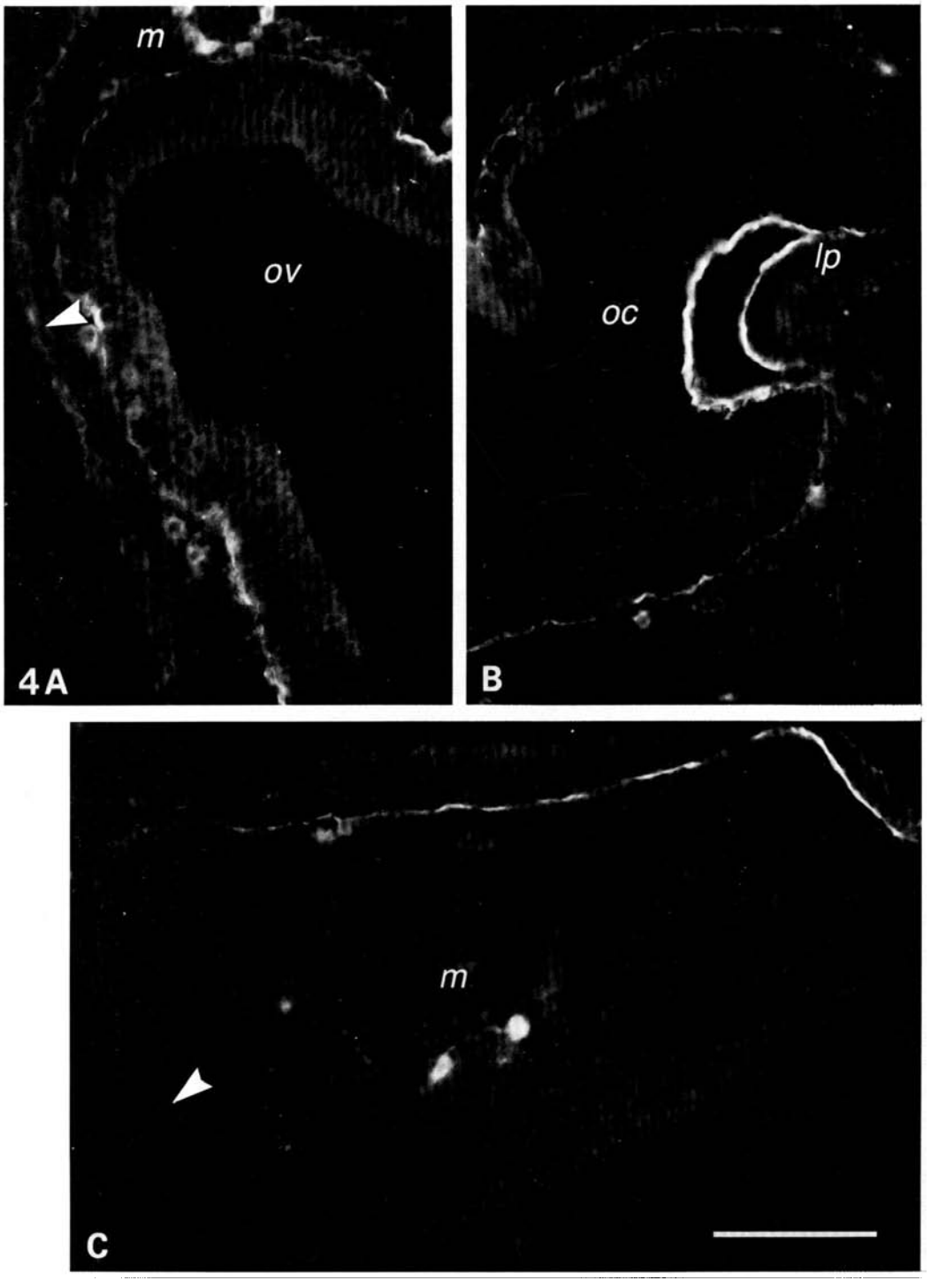
Sey^H has been mapped to chromosome 2. In a cross with *non-agouti* (*a*), Sey^H gave a recombination percentage of $25·8 \pm 3·5\,\%$ (Table 3). The marked deficiency in $Sey^H/+$ offspring mentioned above is clear in the data. In the five point cross shown in Table 3, the most probable position of Sey^H is proximal to *pallid* (*pa*) since this gives the lowest number of double recombinants. In this cross the recombination percentage with *a* is only $8 \pm 2·2\,\%$; the reason for the discrepancy with the Sey^H-*a* value is not known.

Further evidence that *Sey* maps proximal to *pa* comes from its linkage with *mahogany* (*mg*) which gave a value of $12·4 \pm 2·4\,\%$ (Table 3). The locus of *mg* is close to that of *we*.

DISCUSSION

The data presented here suggest that the effect of the *Sey* mutation is limited specifically to a defect in the growth and differentiation of the presumptive lens and nasal placodes. Homozygous embryos lack eyes and noses and, although they develop to term, they die soon after birth, presumably because of inability to breathe since, unlike humans, mice cannot breathe through the mouth. Unless precautions are taken to separate mother and young at birth the homozygotes are normally eaten and this accounts for why they have not previously been identified.

Although we are still a long way from identifying the primary site of action of the *Sey* mutation, several possible mechanisms can be eliminated with some confidence. Firstly, the mutation does not affect all of the cephalic placodes, since the sensory ganglia V and the otic rudiments apparently develop normally (Fig. 2). Couly & Le Douarin have recently proposed from chick–quail chimaera studies that the adenohypophysis and intermediate pituitary are derived from the anterior neural ridge (Couly & Le Douarin, 1985). If such an anterior pituitary placode exists in mammals it is also apparently unaffected by the *Sey* mutation. The fact that the otic vesicles and whisker and tooth rudiments are normal (Fig. 2, and data not shown) also argues against a defect in the cytoskeleton or intercellular organization of ectodermal cells required for the morphogenetic movements of invagination or vesicle formation. Finally, the distribution of laminin in the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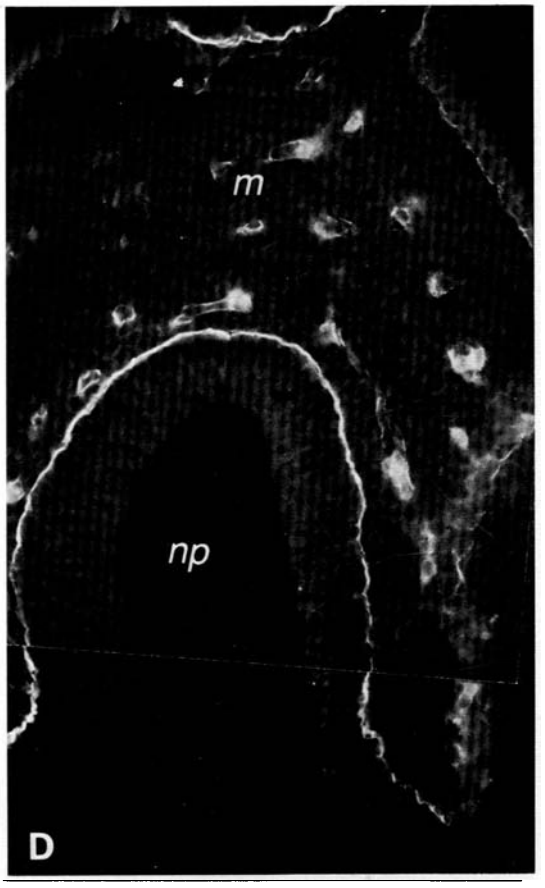

Fig. 4. Immunofluorescent localization of laminin in *Sey/Sey* embryo and littermate at 10·5 days *p.c.* Panels A–D show horizontal sections, from levels corresponding approximately to those shown in Fig. 2A and B, of one *Sey/Sey* embryo (A,C) and one *Sey/+* or +/+ littermate (B,D).

(A) *Sey/Sey* embryo showing laminin immunoreactivity associated with the basement membrane separating the optic vesicle (*ov*) from the mesoderm (*m*). Note absence of contact between the vesicle and the head ectoderm (arrow).

(B) Littermate showing intense laminin immunoreactivity associated with basement membranes lining the opposing faces of the optic cup (*oc*) and lens placode (*lp*).

(C) *Sey/Sey* embryo showing laminin immunoreactivity associated with basement membranes around brain and separating ectoderm (arrow) from mesoderm (*m*) in the region corresponding to (D).

(D) Littermate showing intense laminin staining in basement membranes separating the nasal pit (*np*) from mesodermal tissue (*m*) with blood vessels.

Scale bar, 0·1 mm.

cephalic region of homozygous embryos (Fig. 4) suggests that a deficiency or excess of this extracellular matrix component is not a primary causative factor. We cannot, of course, exclude the possibility that in the mutant the laminin, or some other component of the extracellular matrix, has undergone abnormal post-translational modification affecting its normal function in morphogenesis, but such an effect would have to be specific to the lens and nasal placodes.

Table 2. *Phenotype of embryos resulting from* (Sey/+ × Sey^{H}/+) *and* (Sey^{H}/+ × Sey/+) *crosses**

Total number of implantation sites examined	Small implantation sites (moles) or very retarded embryos	Embryos without eyes and nose†	Normal with eyes and nose
81	12	17	52

* Results based on one litter examined at 11·5 days *p.c.*, two at 12·5 days *p.c.*, five at 13·5 days *p.c.* and one at 15·5 days *p.c.* No difference was seen depending on maternal genotype.

† At 13·5 days *p.c.* these embryos were also distinguished by their unfused maxillary processes (see Fig. 4B).

Further investigation is necessary to establish the origin of the frontonasal protrusions seen in *Sey/Sey* and *Sey/Sey*^{H} embryos (Fig. 5). This protrusion may arise from the forward migration of the neural-crest-derived mesenchymal cells that would normally fill the lateral and medial nasal processes (Waterman & Meller, 1973; Le Douarin, 1982; Couly & Le Douarin, 1985).

It is tempting to attribute the failure in lens development in *Sey/Sey* embryos to a defect in the inductive interaction between the optic vesicle and the overlying ectoderm since these tissues apparently fail to make direct contact (Figs 2–4). However, experiments with amphibian embryos have shown that lens induction is a complex and multistage process (reviewed by Jacobson, 1966; McAvoy, 1980; Nieuwkoop *et al.* 1985). It is initiated at the early gastrula stage and involves

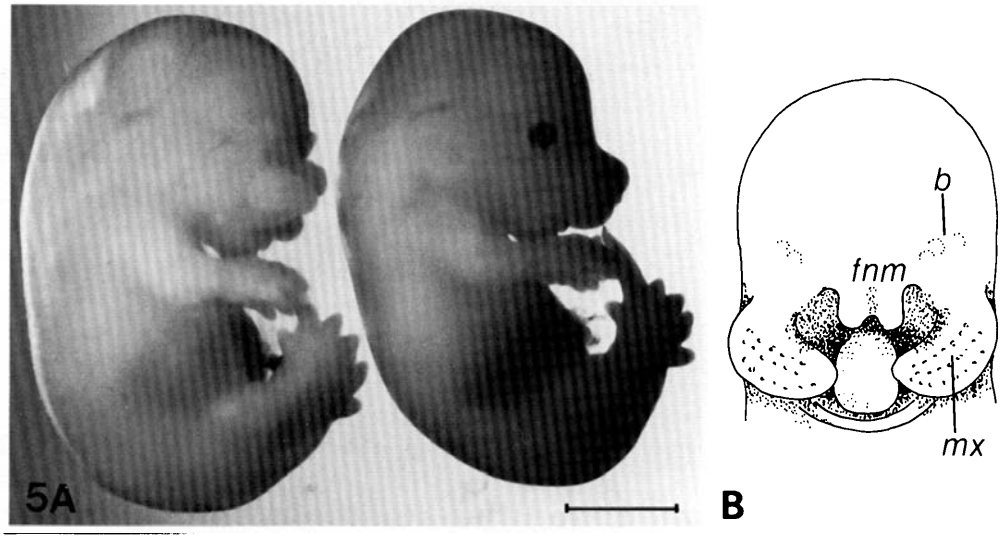

Fig. 5. External morphology of *Sey/Sey*^{H} embryo and littermate at 13·5 days *p.c.*

(A) Photomicrograph of littermate from a cross *Sey*/+ × *Sey*^{H}/+). A double heterozygous *Sey/Sey*^{H} embryo on the left.

(B) Line drawing of head region of the *Sey/Sey*^{H} embryo showing absence of eyes and nose and retarded facial morphogenesis. *mx*, maxillary process with whisker rudiments; *fnm*, frontonasal mass or protruberance; *b*, small blebs (not always observed).

Scale bar, 2·5 mm in A and 1·25 mm in B.

Table 3. *Position of* Sey *and* Sey^H *on chromosome 2*

(A) Cross	*Sey*^{H}+	*Sey*^{H}*a*	++	+*a*	Total	Recombinants	
Sey^H+/+*a* × +*a*/+*a*	24	6	28	72	130	34	41/159 = 25·8 ± 3·5 %
Sey^H*a*/++ × +*a*/+*a*	3	5	17	4	29	7	

(B) Cross	*Sey*+	*Sey mg*	++	+*mg*	Total	Recombinants	
Sey+/+*mg* × +*mg*/+*mg*	113	16	15	105	249	31	31/249 = 12·4 ± 2·1 %

(C) Cross $\frac{Sey^H\ +\ +\ +\ +}{+\ pa\ we\ un\ a^t} \times \frac{+\ pa\ we\ un\ a^t}{+\ pa\ we\ un\ a^t}$

Region	1	2	3	4	Number of mice
Non-crossover	*Sey*^{H}	+	+	+	46
	+	*pa*	*we*	*un*	*a*^{t} 91
Crossover region					
1	+	+	+	+	3
	Sey^H	*pa*	*we*	*un*	*a*^{t} 0
2	+	*pa*	+	+	1
	Sey^H	+	*we*	*un*	*a*^{t} 0
3	+	*pa*	*we*	+	3
	Sey^H	+	+	*un*	*a*^{t} 0
4	+	*pa*	*we*	*un*	+ 3
	Sey^H	+	+	+	*a*^{t} 2
Double 1 and 3	+	+	+	*un*	*a*^{t} 1
					Total 150

Region	Recombination	%
1	4/150	2·67 ± 1·32
2	1/150	0·66 ± 0·66
3	4/150	2·67 ± 1·32
4	5/150	3·33 ± 1·47
Sey^H-*a*^{t}	12/150	8·0 ± 2·22
pa-*a*^{t}	10/150	6·67 ± 2·04

interaction between the presumptive lens placode and, sequentially, the endoderm and the heart mesoderm. It is only in the late neurula stage that interaction between the presumptive placode and the optic vesicle triggers the final stages of lens development, culminating in the invagination of the ectoderm. A similar series of events is involved in the induction of the amphibian nasal placode (see references above). It is not yet known whether a comparable sequence occurs during mammalian development. If it does, then the *Sey* mutation could interfere with lens and nose development during any of the phases, including the earliest inductive interaction between the endoderm and the anterior neuroectoderm. Failure to establish or maintain contact between ectoderm and optic vesicle would then be a consequence, rather than a cause of the mutant phenotype. The idea that the *Sey* mutation could act early gains some support from the observation that alcohol administration to pregnant mice at 7 and 8 days *p.c.* (i.e. well before differentiation of the cephalic placodes) produces microphthalmia and defects in nasal pit development analogous to the foetal alcohol syndrome in humans (Sulik & Johnston, 1983; Webster, Walsh, McEwen & Lipson, 1983).

More detailed examination of the morphogenesis of the optic vesicle in *Sey/Sey* embryos may throw light on the significance of the mesodermal cells between the optic vesicle and overlying ectoderm (Fig. 3). Persistence and proliferation of mesodermal cells in this region have been reported in homozygous *ey-1/ey-1* embryos by Silver & Hughes (1974), but not by others (Harch, Chase & Gonsalves, 1978).

We have shown here that the *Sey* mutation, which arose spontaneously in a colony in Edinburgh (Roberts, 1967), is allelic with a radiation-induced mutation, now designated Sey^H. However, the two alleles are clearly not identical. Heterozygous $Sey^H/+$ are more severely affected than *Sey*/+ in that they are small, have belly spots and reduced viability, and the eyes have coloboma in addition to being small. In addition, the compound Sey^H/Sey embryos are more severely affected than *Sey/Sey*, and homozygous Sey^H/Sey^H appear on the basis of limited data to die early in gestation. The small size and reduced viability of heterozygotes, and early death of homozygotes are consistent with Sey^H being a small deletion covering adjacent loci, and this in turn is consistent with its origin among the offspring of an irradiated female. Sey^H maps to chromosome 2 of the mouse with a linkage of between 8 and 25·8% with non-agouti (*a*) (Table 3). A value of $12 \pm 3{\cdot}3\%$ between *Sey* and *a* was obtained by Day (1971) with 99 animals. Two other genes, with similar effects on the eye in heterozygotes, have also been mapped to chromosome 2, at roughly similar distances from *a*. These are coloboma (*Cm*) (Searle, 1966) and Dickie's small eye (*Dey*) (Theiler, Varnum & Stevens, 1978). *Dey*/+ resembles $Sey^H/+$ in having small body size and white belly spots, in addition to small eyes and coloboma, and shows 15·34% recombination with *a* (Theiler *et al.* 1978). *Cm*/+ shows the small eyes and coloboma also seen in $Sey^H/+$ and has been mapped proximal to *a* (Davisson & Roderick, 1981) between *we* and *un*. In a five point cross, the most probable position of *Sey* is proximal to pallid (*pa*) (Table 3) and this location is consistent with the linkage with *mahogany*.

However, the data do not exclude the possibility that *Cm* and *Sey* are in fact at the same point, and in view of the phenotypic similarity of the heterozygotes, allelism tests between *Sey*, *Cm* and *Dey* would be very valuable. In any case, *Sey* maps to a comparatively well-defined region of chromosome 2 which includes at least two genes, *limb deformity* (*ld*) and *Strong's luxoid* (*lst*) that affect embryonic development (Green, 1981; Forsthoefel, 1963; Woychik *et al.* 1985). The region also includes cloned genes (e.g. β-2-microglobulin) which may be useful for mapping the *Sey* locus more precisely.

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