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# *Small eye* (*Sey*): a mouse model for the genetic analysis of craniofacial abnormalities

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## Summary

***Small eye* (*Sey*) is a dominant mutation in the mouse affecting the embryonic development of the eyes and nose. In homozygous *Sey/Sey* embryos, the optic vesicles grow out but there is no lens induction and the nasal pits fail to develop. Scanning electron microscope studies of *Sey/Sey* embryos show that the maxillary processes develop normally and fuse with ridges of ectoderm in the frontonasal position. In *Sey*/+ heterozygotes, the vacuolated lens is smaller than normal, and there is folding of the margins of the optic cup and ingrowth of mesodermal cells. Evidence is presented that *Sey* is not allelic with *Coloboma* (*Cm*), another mutation affecting eye development on chromosome 2.**

Key words: *Small eye* (*Sey*), eye, nose, developmental mutation, optic vesicle, lens placode, nasal placode.

## Introduction

It will be clear to readers of this volume that the morphogenesis of the vertebrate head involves a complex series of reciprocal interactions between different cell populations (e.g. ectoderm, neurectoderm, cranial paraxial mesoderm and neural crest). These are brought together as a result of cell migration and the coordinated growth and fusion of facial primordia (see Wedden *et al.*, this volume). A variety of biochemical, molecular and immunological techniques are being used to identify factors responsible for mediating the various subroutines of this overall programme. Another very powerful tool that can be brought into play is that of genetics, and a number of mouse mutants have been described which show inherited craniofacial abnormalities (Green, 1981). Some of these appear to be the result 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). However, other mutations seem to have more specific effects on cell interactions during cranial morphogenesis. One of these is the dominant mutation known as *Small eye* (*Sey*) which has been mapped to mouse chromosome 2, about 5 cm proximal to the b-2-microglobulin locus (Hogan *et al.* 1986). Heterozygous *Sey*/+ mice have small vacuolated lenses and develop cataracts within a few weeks of age. Homozygous *Sey/Sey* embryos can be distinguished as early as 10·5 days *post coitum* (*p.c.*) by the complete failure of lens induction and the absence of nasal pits. Subsequently the optic vesicles become distorted and degenerate, and nasal cavities and olfactory bulbs do not develop. Homozygous embryos die soon after birth because newborn mice cannot breathe through their mouths. Apart from these defects in eye and nose development, *Sey/Sey* embryos appear quite normal, suggesting that the *Sey* mutation affects some specific aspect of craniofacial morphogenesis, rather than a process common to many embryonic tissue interactions.

In this paper, we describe in more detail the embryonic development of *Sey/Sey* and *Sey*/$^{+}$ embryos, and speculate about the primary defect. We show that while *Sey* is allelic with *Dickie small eye* (now *Sey*$^{Dey}$) and Harwell *small eye* (*Sey*$^{H}$), it is not

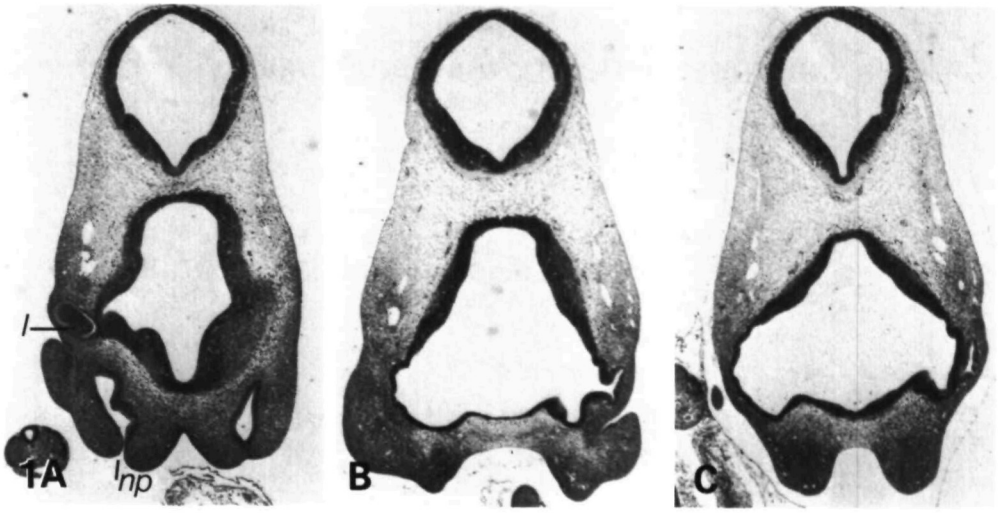


**Fig. 1.** Internal morphology of two *Sey/Sey* embryos and one Sey/+ or +/+ littermate at 11·5 days *p.c.* Embryos were fixed in Bouin's fixative, embedded in paraffin wax and serially sectioned at 7 μm. Sections were stained with haematoxylin and eosin. (A) *Sey/+* or +/+ embryo, showing eye cups with lens (*l*) and nasal pits (*np*). (B,C) *Sey/Sey* embryos sectioned at the same level, showing the absence of lens and nasal pits.

allelic with *Coloboma* (*Cm*), another mutation affecting eye development on chromosome 2 (Searle, 1966).

A colony of *Sey/*$^{+}$ mice is maintained at the National Institute for Medical Research. They are currently at the 6th backcross to C57BL/10ScSn. *Sey/*$^{+}$ mice are clearly distinguished soon after birth by the size of their eyes and develop cataracts after 3 weeks. On the C57BL background about 25% (25/96) of the *Sey/+* offspring of both sexes develop hydrocephaly and die by the about 8 weeks of age. Histological examination of fixed brains suggests bilateral hydrocephaly of the lateral ventricles, not involving the third ventricle. *Cm/+* males were kindly provided by Dr J.-L. Guenet, Institut Pasteur. For the timing of embryos, noon on the day of the vaginal plug is 0·5 days *p.c.*

## Morphology of *Sey/Sey* embryos

Previous studies showed that, in presumed *Sey/Sey* embryos, the optic vesicles grow out but lens induction fails to take place (Hogan *et al.* 1986). Mesodermal cells are present between the optic epithelium and the overlying ectoderm. However, it is not clear whether this is due to failure of mesodermal cells in this position to die, thus preventing intimate contact between optic vesicle and presumptive lens placode (Silver & Hughes, 1974 and see Bard *et al.*, this volume), or whether the mesodermal cells migrate into the space made available as a result of the primary failure of the two epithelial tissues to make or maintain intimate contact.

Absence of contact between optic vesicle and ectoderm is apparent in embryos at 10·5 days *p.c.* (Hogan *et al.* 1986). By 11·5 days *p.c.* the optic vesicle has become very distorted, as shown in Fig. 1. The sections also show the absence of nasal pits in the homozygous embryos.

Scanning electron microscopy confirms our previous observations that the maxillary, mandibular and hyoid processes develop normally in presumed *Sey/Sey* embryos (Fig. 2). At 11·5 days *p.c.*, it can be seen that the maxillary processes fuse with small protrusions in the position of the frontonasal mass (Fig. 2B,D). These protrusions would normally be greatly enlarged and expanded by the growth of the nasal pits and the surrounding neural-crest-derived mesenchyme cells (Fig. 2A,C).

## Morphology of the eyes of *Sey/+* embryos

Fig. 3 shows a section through the eye of a presumed heterozygous *Sey/+* embryo at 15·5 days *p.c.* The lens is vacuolated and is only about half the size observed in normal littermates. Probably because of the small size of the lens, there is infolding of the anterior margins of the eye cup and infiltration of mesodermal cells.

## *Sey* is not allelic with *Coloboma* (*Cm*)

In previous studies, we have shown that the *Sey* mutation maps to chromosome 2, about 5 cm from the beta-2-microglobulin locus. *Sey* is allelic with two

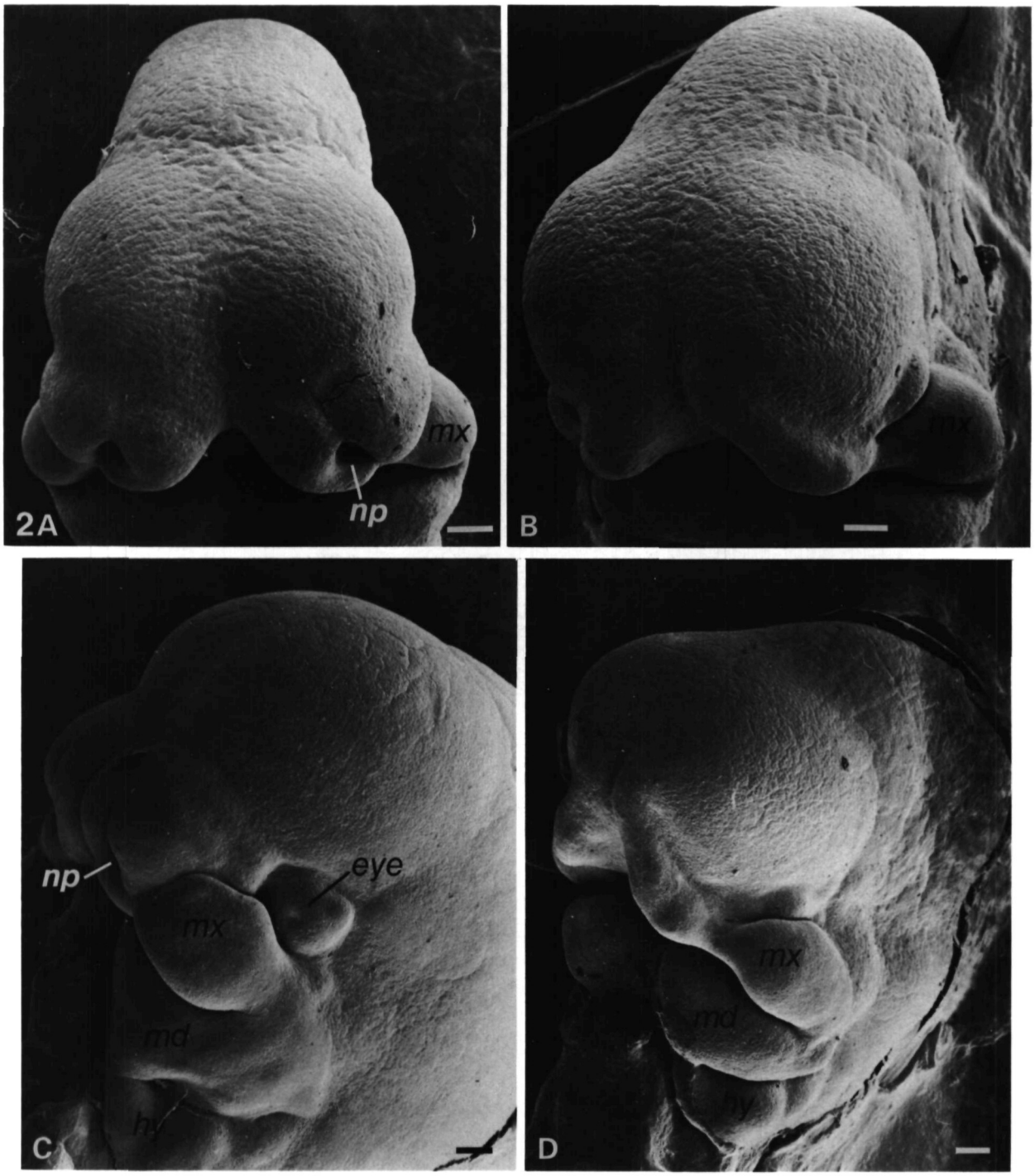


**Fig. 2.** Scanning electron micrographs of *Sey/Sey* and *Sey/+* or *+/+* littermates at 11·5 days *p.c.* Embryos were fixed with 2 % glutaraldehyde/1·5 % paraformaldehyde in 0·1 M-sodium cacodylate, pH 7·25 overnight and postfixed in 1 % osmium tetroxide. After sequential ethanol dehydration they were transferred via acetone to $CO_2$ and critical-point dried in a Samdri 780. They were then sputter coated with 7 nm gold and viewed in a JSM 35 CF scanning electron microscope. (A,C) *Sey/+* or *+/+* embryos, showing well-developed nasal pits (*np*), maxillary (*mx*), mandibular (*md*) and hyoid (*hy*) processes and surface of the eye (*eye*). (B,D) *Sey/Sey* embryos, showing a 'ridge' of tissue in the frontonasal region and the absence of nasal pits and eye. Scale bar, 100 μm.

early prenatal lethal mutations, $Sey^{H}$ and $Sey^{Dey}$ (Dickie's small eye), both of which may involve small deletions or DNA rearrangements at the *Sey* locus but extending into nearby genes which affect early development (Hogan *et al.* 1986; Hogan, Hetherington & Lyon, 1987).

Since the mutation *Coloboma* (*Cm*) has been mapped to mouse chromosome 2, proximal to *non-*

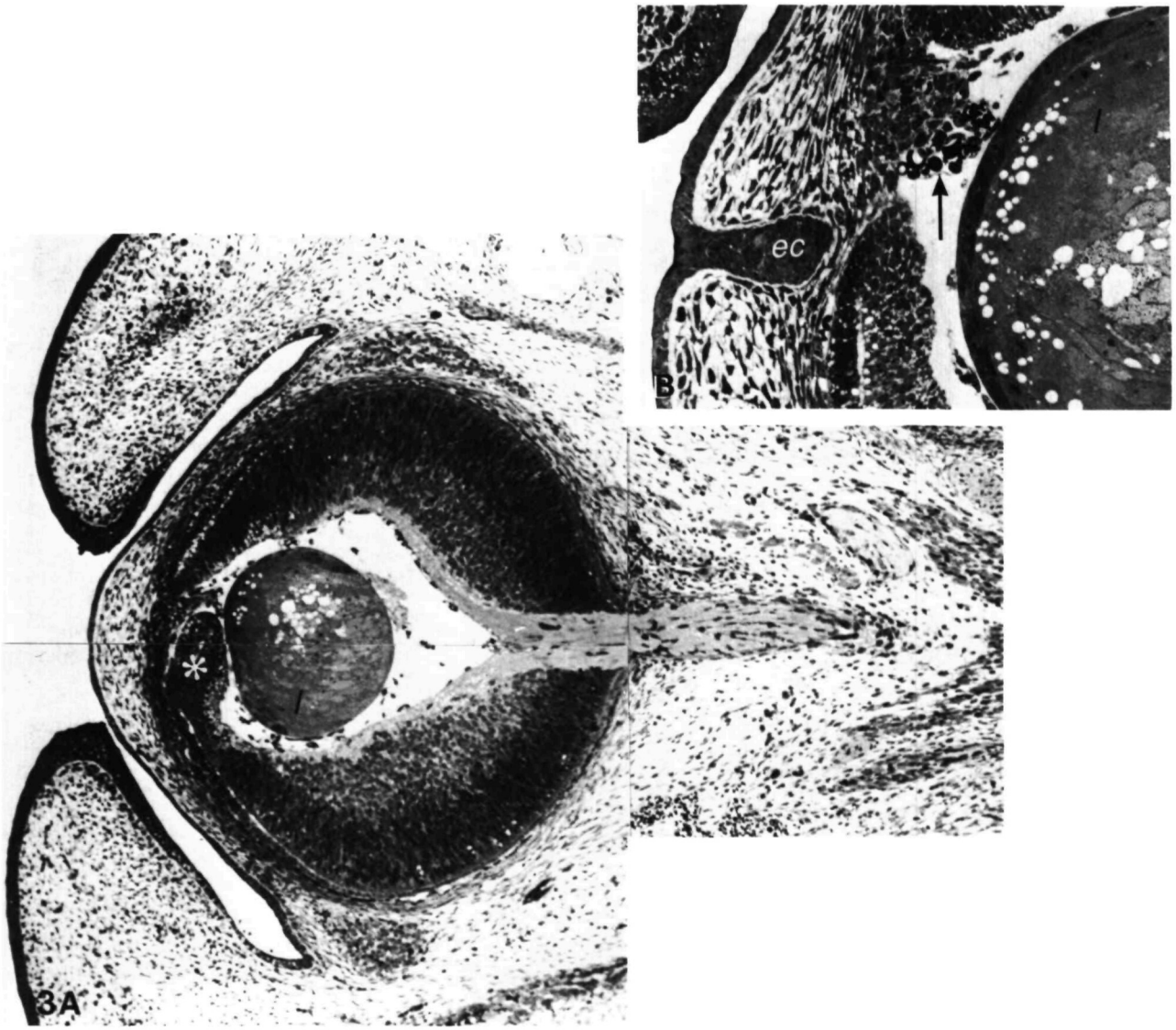


**Fig. 3A.** Section through the eye of a presumed heterozygous *Sey*/+ embryo at 15·5 days *p.c.* The dorsoventral diameter of the lens (*l*) (250 μm) is half that seen in normal littermates and the lens cells are vacuolated. Note the abnormal folding of the margins of the eye cup in front of the lens (*). Insert B. Another section through the same eye showing remnant of ectoderm (*ec*) possibly due to the incomplete separation of the lens. Note also the abnormal infiltration of mesoderm into the eye cup (arrow).

*agouti* (a), between *we* and *un* (Searle, 1966; Davisson & Roderick, 1981), we tested for allelism between *Sey* and *Cm*. This was achieved by crossing *Sey*/+ heterozygous females with *Cm*/+ males. Homozygous *Sey*/*Sey*, *Sey*/*Sey*$^{Dey}$ or *Sey*/*Sey*$^{H}$ embryos have a characteristic phenotype, lacking both eyes and nose (Hogan *et al.* 1986; Hogan, Hetherington & Lyon, 1987). However, as shown in Table 1, only 2/68 embryos from the cross between *Sey*/+ and *Cm*/+ lacked eyes altogether, and these did not resemble *Sey*/*Sey* embryos in other respects. Rather, the results are compatible with *Sey* and *Cm* being nonallelic and with both *Sey*/+ and *Cm*/+ embryos having colobomatous eyes, so that they cannot be clearly distinguished by external observation at the stages examined, and *Sey*/*Cm* embryos being microphthalmic.

## Discussion

There are several possible explanations for the underlying defect caused by the *Sey* mutation. First, there may be a failure in the mechanism whereby regions of the ectoderm of the early embryo become specified as presumptive lens or nasal placodes, programmed to respond later to inductive signals from either the lens vesicle or the frontonasal mesenchyme, respectively. According to this hypothesis, in *Sey*/*Sey* embryos, no

**Table 1.** *Phenotype of embryos resulting from crosses between* (Sey/+) ♀ *and* (Cm/+) ♂*s*

| Age (days *post coitum*) | Total number of implantation sites | Moles, or very retarded embryos | Embryos with normal eyes (symmetrical pigmentation) and noses | Embryos with colobomatous eyes (asymmetrical pigmentation) and normal noses | Embryos with very small eyes (dot of pigment) and normal noses | No eyes or nose |
|---|---|---|---|---|---|---|
| 13½ d | 10 | 1 | 7 | 2 | – | – |
| 14½ d | 11 | 2 | 1 | 6 | 2 | – |
| 15½ d | 10 | – | 3 | 4 | 3 | – |
| 16½ d | 8 | – | 1 | 6 | 1 | – |
| 17½ d | 19 | 3 | 2 | 8 | 4 | 2 |
| 18½ d | – | 3 | 3 | 3 | 4 | – |
| | 68 | 6 | 17 | 29 | 14 | 2 |

positional information is given at all, while in heterozygotes the areas instructed to become lens or nasal placode are abnormally small. Alternatively, the defect could be in the process of induction itself, attenuating the production, transfer or reception of the signal which presumably passes from one tissue to another. These models assume that the *Sey* mutation involves a dominant 'loss of function' rather than 'gain of function', but other scenarios cannot be excluded. The most fruitful way to distinguish between these models is to identify the normal *Sey* gene product by means of 'reverse genetics', i.e. cloning the gene, identifying the transcript and predicting the nature of the gene product. The fortuitous localization of the *Sey* mutation close to the beta-2-microglobulin locus and to other cloned loci (e.g. Woychik *et al.* 1985), and the existence of a number of presumed deletion mutants at the *Sey* locus may allow this goal to be achieved in the not-too-distant future.

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