

# Association of Megacolon with Two Recessive Spotting Genes in the Mouse

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MUTANT genes causing pathological conditions in mice comparable to similar conditions in man are potentially of great value. Such mutations as dystrophia muscularis (*dy*)<sup>3</sup>, obese (*ob*)<sup>5</sup>, dwarf (*dw*)<sup>9</sup>, the dominant spotting alleles (*W*, *W'*)<sup>4</sup> with their accompanying anemias, and others have been extensively used. In 1957 Derrick and St. George-Grambauer<sup>5</sup> reported the appearance of megacolon in mice. The incidence in their colony was approximately 3.2 per thousand and no specific association with coat color was noted. Histological studies revealed that myenteric ganglion cells were absent from the lower colon, a condition similar to that found in Hirschsprung's disease in man. In 1960 Bielschowsky and Schofield<sup>1</sup> reported an incidence of megacolon of 10 percent in mice of the piebald (*s/s*) NZY strain. The association of deficiency of myenteric ganglion cells with deficiency in hair pigmentation is interesting from an embryological point of view since both the pigment cells of the hair and the nerve cells of the colon are derived from cells that migrate from the neural crest early in embryonic life.

Recently a new recessive lethal allele of piebald spotting has appeared. This mutation, called piebald-lethal (symbol *s'*) causes more white spotting than *s* and all homozygous mice die with megacolon.

## Genetics of Piebald-Lethal

The mutant was found in April, 1959, at the Jackson Laboratory in the F<sub>2</sub> generation from a cross between a C3H/HeJ female mouse showing a head blaze and belly spot and a C57BL/6J male. All six F<sub>1</sub> offspring were normal in appearance, except that one male had a small belly spot. The first F<sub>2</sub> litter contained 10 offspring, eight normal or full colored mice and two black-eyed white-coated mice showing a few small patches of pigmented hair about the ears, eyes, and tail. It was noted that the two black-eyed white mice (hereafter called piebald-lethals, *s' s'*) in this litter were dead by 9 and 14 days of age. In the next litter from the same parents three piebald-lethals were present and five full-colored siblings. Of the three *s'/s'* mice one was dead by day 6 and the

other two by day 12. These deaths suggested that the gene was lethal in its action. Further breeding pairs were made up from the normal offspring in these and subsequent litters. The results from all breeding pairs known to be heterozygous for piebald-lethal (*s'/+*) are given in Table I, cross 1.

A total of 2545 F<sub>2</sub> mice were raised; 1987 full-colored and 558 piebald-lethals. This ratio differs significantly from the expected 3:1 ratio for a single recessive gene ( $\chi^2 = 12.84$ ). However, the deficiency of *s'/s'* mice can probably be explained by death of some of these animals prior to classification. Two litters were eventually produced from a mating of an *s'/s'* male to an *s'/s'* female and all 13 offspring were *s'/s'* as expected for a recessive gene. The results of this and all other crosses are given in Table I.

The first *s'/s'* male to live and breed was mated to a homozygous piebald (*s/s*) female from a multiple recessive stock (cross 3). All offspring from this cross were heavily spotted and looked like *s/s* mice. To test the hypothesis that *s* and *s'* were alleles and that the spotted mice produced from cross 3 were not the result of interaction of mutant alleles at two loci each in the heterozygous state, F<sub>1</sub> mice were mated *inter se* and all offspring were classified. If there were two loci not closely linked, wild-type offspring would appear as one of the three expected classes in a ratio of  $\frac{5}{16}$  wild type,  $\frac{1}{16}$  spotted, and  $\frac{1}{16}$  piebald-lethal. If there was only one locus or two closely linked loci the offspring would be of two classes,  $\frac{3}{4}$  spotted and  $\frac{1}{4}$  piebald-lethal. The results (Table I, cross 4) show two classes only, 577 spotted and 136 piebald-lethal, and indicate allelism or close linkage. The shortage of *s'/s'* mice ( $\chi^2 = 13.35$ ) is again probably due to death before classification. The similar phenotypic effects of *s* and *s'* favor the hypothesis that they are alleles rather than mutants at closely linked loci.

Further testing of the one locus vs. two locus hypotheses was carried out by outcrossing F<sub>1</sub> (*s/s'*) male mice to C57BL/6J females. Any spotted mice appearing among the offspring of this cross would have to result from recombination between alleles at two different loci. All 228 F<sub>1</sub> offspring (Table I, cross 5) were wild type, thus confirming the conclusion that *s'* is an allele of *s*.

## Megacolon in *s' s'* and *ls ls* Mice

The unfailing association of megacolon with the black-eyed white-coated phenotype of *s'/s'* mice is

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useful in studying the development of megacolon since all animals that will eventually have megacolon are clearly distinguishable at 2 to 3 days of age by a lack of skin pigmentation. Megacolon is grossly evident in weaning-age or older mice at autopsy by a markedly distended colon which is filled with hard fecal matter, generally extending from the caecum to varying distances above the rectum. Very young (2 to 15 day-old) mice may or may not show gross evidence of megacolon. When they do, their colons are distended and full of soft gelatinous fecal material in which the beaded appearance of early pellet formation is not present as it is in normal mice of this age.

Mice of the  $s^1/s^1$  genotype die as early as 1 to 2 days after birth or as late as 15 months, with the usual age of death approximately 15 days. To date 13  $s^1/s^1$  mice, nine females and four males, have lived to breed but all have eventually died with megacolon between 3 and 15 months of age.

Of the 44  $s/s^1$  mice raised from cross 3, 12 were used as breeders for cross 4 and of these 12, three died with megacolon at 11, 13, and 23 months respectively. The others were free of megacolon when autopsied between 12 and 23 months of age.

In order to determine if myenteric ganglion cells were deficient in the colons of young  $s^1/s^1$  mice, histological preparations were made of the colons from six piebald-lethal and six normal siblings ( $s^1/+$  or  $+/+$ ); one mutant and normal pair at 2 days, two pairs at 3 days, one pair at 6 days, and two pairs at

12 days. The entire large intestine including caecum and rectum were filled with Bouin's solution via a syringe, removed intact, and placed in an extended position in fresh Bouin's solution. After fixation each colon was cut into four sections each approximately 10 mm in length. The most distal section was numbered 1 and the most proximal, 4. Longitudinal serial sections were cut at  $8\ \mu$  and stained with hematoxylin and eosin. The results were similar to those reported by other investigators<sup>1,2</sup> for older mice with megacolon. In the normal mice at all four ages, groups of myenteric ganglion cells of varying size were present approximately every 50-100  $\mu$  in all four sections of the colon from rectum to caecum. There were few to no ganglion cells evident in the first or posterior 10 mm section of the colons of  $s^1/s^1$  mice at any of the ages studied. A gradual increase in the number of ganglion cells was evident in the next two sections. The fourth or proximal 10 mm section of colon in the  $s^1/s^1$  mice was roughly comparable in number of ganglion cells to the same section in the normal controls. This pattern of absence and then gradual increase in number of ganglion cells from the distal to the proximal end of the colon in piebald-lethal mice was the same for each age. The data are tabulated in Table II.

Another recessive spotting gene in the mouse not allelic with  $s$  also causes megacolon in homozygotes. This gene, called lethal spotting ( $ls$ )<sup>7</sup>, resembles  $s$  in its effect on coat pigmentation except that the ears

Table I. Results of matings of piebald-lethal

Cross	Mating	Offspring			Total	$\chi^2$
		Normal (+-)	Black-eyed white ( $s^1/s^1$ )	Spotted ( $s/s^1$ or $s/s$ )		
1	$s^1/+ \times s^1/+$	1987	558	0	2545	12.84
2	$s^1/s^1 \times s^1/s^1$	0	13	0	13	—
3	$s/s \times s^1/s^1$	0	0	44	44	—
4	$s/s^1 \times s/s^1$	0	136	577	713	13.35
5	$+/+ \times s/s^1$	228	0	0	228	—

Table II. Estimated number of ganglion cells in sections of colons of  $s^1/s^1$  and  $ls/ls$  mice and their normal sibs\*

Age in days	$s^1/s^1$				$+/+ \text{ or } s^1/+$				$ls/ls$				$+/+ \text{ or } ls/+$			
	1	2	3	4†	1	2	3	4	1	2	3	4	1	2	3	4
2	0	+	++	+++	+++	+++	+++	+++								
3	0	+	++	+++	+++	+++	+++	+++								
6	0	+	++	+++	+++	+++	+++	+++								
12	0	+	++	+++	+++	+++	+++	+++	0	++	++	+++	+++	+++	+++	+++
12	0	+	++	+++	+++	+++	+++	+++	0	++	++	++	+++	+++	+++	+++

\* 0 = ganglion cells absent.  
 + = number of ganglion cells very reduced.  
 ++ = number of ganglion cells reduced.  
 +++ = number of ganglion cells normal.  
 † 10 mm sections of colon numbered from distal end, i.e., 1 most distal, 4 most proximal.

and tails of *ls/ls* mice are less pigmented than those of *s/s*. A few *ls/ls* mice have lived to breed but all eventually die with megacolon.

Histological preparations like those made from *s<sup>1</sup>/s<sup>1</sup>* mice and controls were made from the colons of two *ls/ls* mice and two normal siblings (*ls/+* or *+/+*) at 12 days of age in order to determine if a deficiency of myenteric ganglion cells was also associated with megacolon in this mutant. The results were the same as those for piebald-lethal mice, with the most distal of the four sections of colon clearly aganglionic and the most proximal section approximately normal. These data are also included in Table II.

### Discussion

These results, like those of Bielschowsky and Schofield, again demonstrate the striking association between the deficiency of pigment cells in the hair and skin and the deficiency of ganglion cells of the myenteric plexus of the lower colon. Mayer and Maltby<sup>4</sup> have concluded from their studies of pattern development in lethal-spotting mouse embryos that the probable primary site of gene action in *ls/ls* mice is the neural crest. Likewise Mayer<sup>6</sup> has shown that the neural crest plays a determining role in the development of white spotting in *s/s* mice. Yntema and Hammond<sup>10</sup> have produced deficiencies of intrinsic ganglia in the posterior intestines of the chick by incomplete removal of the cervical neural crest. In the light of these experiments and the results reported in this study it seems probable that either a reduction of the number of neural crest cells or a

prevention of the migration of these cells represents the primary action of the mutant genes *s<sup>1</sup>* and *ls*.

### Summary

Hereditary megacolon in mice has been shown to be produced by two different recessive spotting genes, piebald-lethal (*s<sup>1</sup>*) and lethal-spotting (*ls*). Both genes act to reduce the number of pigment cells in the coat and the number of myenteric ganglion cells in the lower colon. Genetic studies with piebald-lethal show that it is an allele of piebald spotting (*s*).

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## Variegated Ovaries in the Pheasant

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IN studies on the effect of irradiation on pheasant (*Phasianus colchicus*) ovaries<sup>1</sup> and studies on the inheritance of the intensity of pigmentation of the plumage etc., it has been revealed that the pheasant ovary possesses varying concentrations of melanocytes surrounding the granular layer of the follicles. They appear to be lodged in the stroma.<sup>2</sup>

From the gross examination of hundreds of pheasant ovaries, it was noted that some of them exhibited a very dark (black) external appearance, Figure 13A. This was especially true of ovaries in very young females from the time of hatching until the yolk formation (deposit) among the early oöcytes. The somewhat older, yolk-laden oöcytes seemingly lost some of the intensely dark color. This may, in part, be due to dilution or rather to the expansion of the accumulating yolk mass and increasing follicle sur-

face. A small percentage, 10-15 percent of the ovaries observed were not intensely black, but were moderately dark or even a light gray. Ovaries without pigmentation, although rare, do occur, Figure 13B. A fairly high percentage of the ovaries were varic-

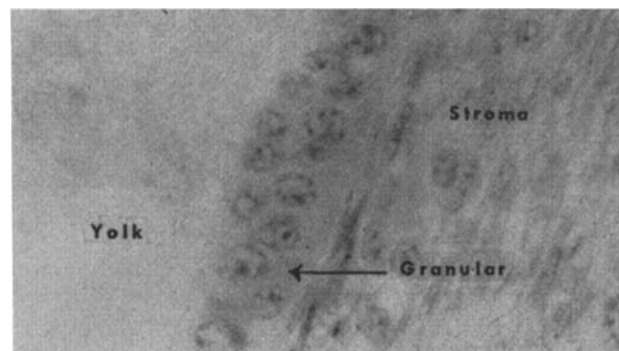


FIGURE 12—Section through an ovarian follicle showing the granular layer and stroma cells; no melanocytes are present.

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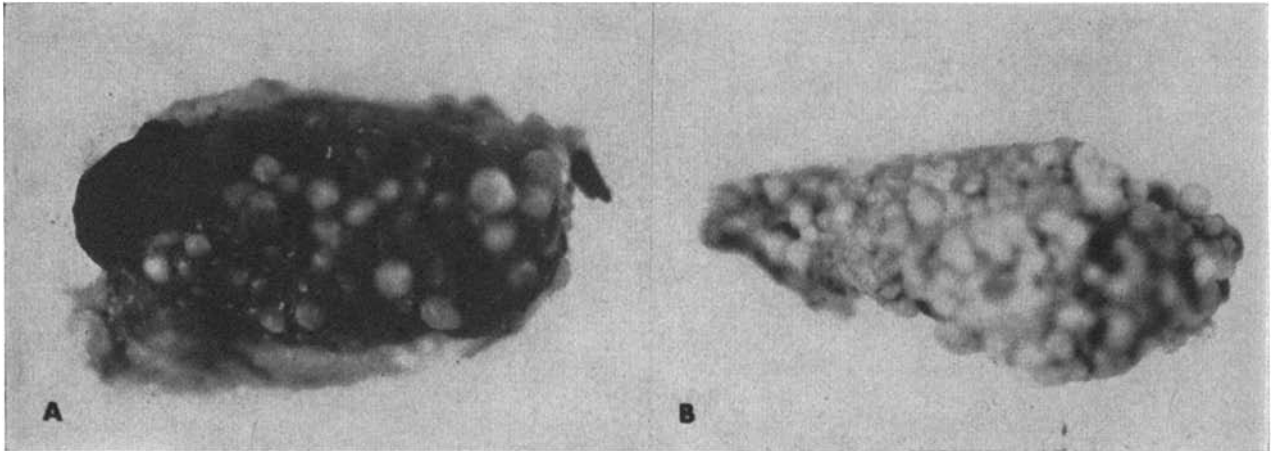


FIGURE 13—A shows a very darkly pigmented ovary; B, an ovary with no pigmentation.

gated or mosaic, with islands of light tissue interspersed with dark. Considerable variation occurred with respect to the size of the colorless and pigmented areas. Again the pigmented areas were intensely black or various shades of gray. The light areas also are often a very light gray instead of "white."

Upon microscopic examination of the very dark ovaries, thick concentrations of melanocytes were observed in the layers surrounding the stratum granulosum, Figure 14A and B. Sparse distribution of pigment cells (Figure 12) was observed in sections of the gray ovaries. As might be expected, gradations from heavy concentrations of pigment cells to an absence of such color-inducing cells was noted.

Sections through the variegated regions of the ovary show very discreet areas of pigment cell concentrations and/or lack of them in adjacent areas.

Gradations in the intensity of the pigment deposition in the plumage, scales, claws, the iris, beak, etc. seem to be correlated with the concentration of the melanocytes surrounding the follicles. Studies carried out thus far indicate that some oocytes are formed in a densely concentrated melanocytic environment. Others develop in follicles with few melanocytes around them and rarely in follicles without any color cells around them. Such differences are possible among different pheasants or in smaller percentages in the same pheasants in cases of the variegated ovary.

Genetic studies being conducted seem to indicate that the plumage color intensity seems to be dependent, in part at least, upon the precursors of melanin in the cytoplasm (yolk) of the egg, which obviously must come from the stroma cells.

The melanocytic cells vary considerably in size from extremely small cells, perhaps with little or no nuclear substance, to large cells with well outlined nuclei. Strong evidence suggests that the cells immediately surrounding the stratum granulosum of the follicle are much more "fragmentary" while the cells lying in stroma some distance away from granular cells are larger and possess more of the typical melanocytic cell structure, Figure 12.

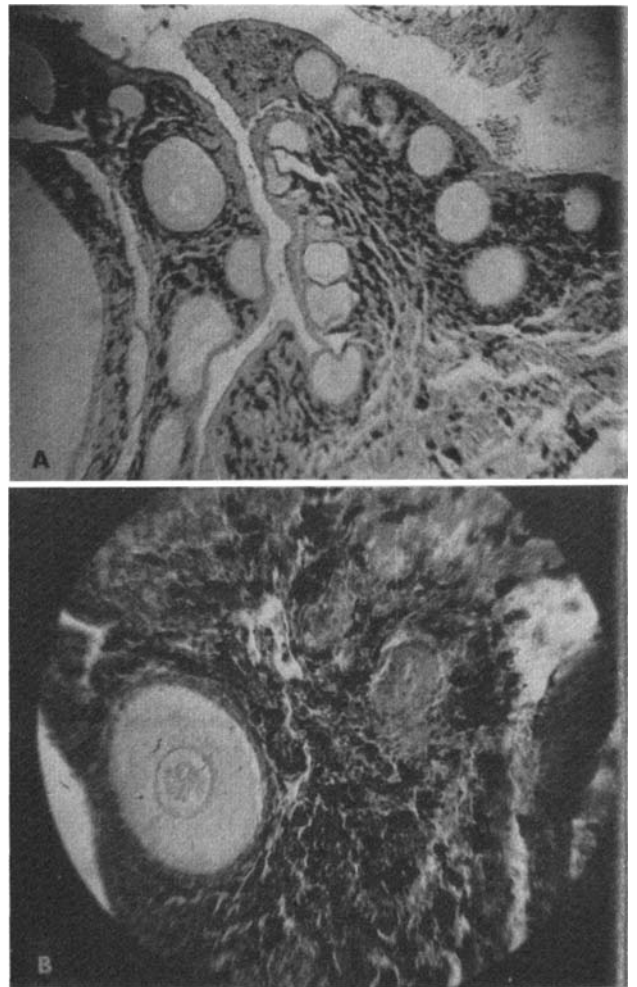


FIGURE 14—A shows ovarian follicles with melanocytes concentrated in the surrounding stroma. B shows a single follicle with surrounding melanocytes.

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