

STEEL, A NEW DOMINANT GENE IN THE HOUSE MOUSE

With Effects on Coat Pigment and Blood

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AGOUTI OF THE C3H STRAIN

Figure 10

Seventeen agouti young of this type appeared in a litter with 22 of the new mutant, Steel.

A SINGLE female of a somewhat diluted fur color and with a small white spot on forehead, belly, and tip of the snout occurred in the eighth generation of brother-sister mating in this laboratory of the inbred C3H strain, obtained from W. E. Heston in 1948. When mated to a wild-type littermate, this female produced 22 (12 ♂, 10 ♀) young similar to herself in appearance and seventeen (9 ♂, 8 ♀) agouti young. The new type was named Steel (*Sl*) and has been maintained in a brother-sister inbred subline of the C3H strain, which may be given the strain symbol C3H *Sl*.

Genetics

Penetrance and Expressivity

Sl is a dominant, as proved by crosses of Steel animals to unrelated C3H

(yielding 121 Steel to 120 agouti), and by outcrosses of Steel to other strains (see below). The good 1:1 ratio from $+/+ \times Sl/+$ and $Sl/+ \times +/+$ crosses within the C3H Sl strain (Table I) indicates that *Sl/+* is normally viable and that penetrance on the C3H background is complete. This latter conclusion also finds support from the tests of 22 agouti offspring of Steel parents (footnotes to Table I): not one of these agoutis was found to transmit Steel.

On the C3H background the Steel phenotype is recognizable by the following externally visible features (see also Figure 11 and compare with Figure 10): (a) slight over-all dilution of the fur color, more extreme on the belly than on the back; (b) light ears, feet, vibrissae, and tail; (c) a white snout tip; (d) almost invariably a small white spot in

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THE STEEL MUTANT

Figure 11

On the C3H background the Steel ($Sl/+$) mutant can be recognized by a slight over-all dilution of the fur color, light ears, feet, vibrissae and tail, a white snout tip and usually a small white spot in the middle of the forehead and on the belly.

the middle of the forehead, and, almost as frequently, one on the belly (however, non-Steel segregants may occasionally, though very rarely, also have a small belly spot); and (e) very occasionally a white blaze between the eyes or a few white hairs on the back. Steels of both sexes are normally fertile and the females make good mothers (Table II).

Steel males were outcrossed to females of six other stocks (C57 Ln, C57 Sp, Rus, $ru/ru;si/si;s/s$, (101 \times C3H) F_1 , $a/a; b/b;pc^{ch}/pc^{ch};dse/dse;s/s$) and 120 young obtained. Of these, 69 were agouti and 51 had somewhat diluted fur color (difference from 1:1 ratio not significant). The fur color dilution was occasionally very slight and was not always accompanied by the pattern of small white spots present in Steels on the C3H background, the head spot being absent most often, the belly spot least often. In view of the occasionally greatly de-

creased expression of Steel, it is possible that the slight (though not significant) excess of agoutis in the outcrosses may represent incomplete penetrance on some backgrounds. The 69 agouti offspring of the outcrosses were not, however, systematically tested to detect such a situation, tests being confined to those few which had small belly spots. (Belly spots in the absence of color dilution were found to be attributable to the background— Ss , or C57BL—rather than the Steel gene.)

Steel by steel matings in the C3HSl strain (Table I) produced no new type and only 63 percent of the mutant type instead of the 75 percent which should have been found on a background where there is complete penetrance (as there is in C3HSl) if the homozygote were viable. This type of mating, moreover, yielded a litter size only 71 percent of normal at birth (Table III). It was, therefore, concluded that $Sl Sl$ animals

TABLE I. Offspring of various types of mating in the C3H Sl strain, classified at weaning age

Type of mating		Steel		Agouti	
♀	♂	♂♂	♀♀	♂♂	♀♀
$+/+$	\times $Sl/+$	218	200	202*	205*
$Sl/+$	\times $+/+$	34	49	38	40
$Sl/+$	\times $Sl/+$	103	110	59†	65†

*Of these 2 ♂♂ 9 ♀♀ tested and found not to transmit Steel.

†Of these 4 ♂♂ 7 ♀♀ tested and found not to transmit Steel.

TABLE II. Comparison between Steel and agouti mothers* with regard to size of litters raised to weaning age

Type of mating		No. of matings	1st litter	2nd & 3rd litters
♀	♂			
$Sl/+$	\times $+/+$	9	5.33	5.85
$+/+$	\times $Sl/+$	9	5.22	5.87

*Mated at comparable times.



DOUBLE HETEROZYGOTE

Figure 12

This type ($SI/+; W^s/+$) is very heavily mottled dorsally and completely white ventrally. The remaining hairs on the back are much lighter than in either $SI/+$ or $W^s/+$.

probably die *in utero*, and dissections were undertaken to verify this point. (Embryology will be discussed below).

Allelism tests with W^s

Because of their general similarity in effect, SI and W^s were tested for allelism. The cross $W^s/+ \times SI/+$ (made in either direction) produced, in addition to agouti, $W^s/+$, and $SI/+$, a new type (Figure 12) which was very heavily mottled dorsally and completely white ventrally. The remaining pigmented hairs on the back were considerably lighter than in either $W^s/+$ or $SI/+$. This new type was assumed to be either

the compound or the double heterozygote. Among 225 F_1 offspring, there were 57 light mottled (Table IV), which is as expected if this type is fully viable. It should be noted that W^s and SI apparently interact, rather than being merely additive, at least with respect to pigment dilution. Light mottled animals of both sexes are fertile.

Under the assumption of allelism, F_2 (light mottled by light mottled) should yield only black-eyed whites and light-mottled animals. However, among 61 F_2 offspring raised, there were 15 which were single heterozygotes and two agouti. This proves SI and W^s to be separate loci.

Light-mottled animals were tested for possible linkage between SI and W^s . In the tabulation (Table IV), $SI/+; +/+$ and $+/+; W^s/+$ progeny were lumped since they usually were phenotypically

TABLE III. Average litter size at birth

Type of mating ♀ × ♂	Number of Mothers	Number of Litters	Average number of young per litter
$+/+ \times SI/+$	28	92	6.62 ± 0.16
$SI/+ \times SI/+$	20	83	4.72 ± 0.20

TABLE IV. Results of crosses between Steel (SI) and Dominant Spotting (W^s)

Mating	Light mottled	$+/+; W^s/+$ and $SI/+; +/+$ ‡	Agouti	Black-eyed white
$+/+; W^s/+ \times SI/+; +/+^*$	57	111	57	—
Lt. mottled × Lt. mottled	27	15	2	17
Lt. mottled × Agouti, repulsion*	119	231	138	—
Lt. mottled × Agouti, coupling*	53	109	60	—

*No significant differences were noted between reciprocal matings.
 †Light mottled F_1 animals were crossed to agouti segregants of the Steel strain.
 ‡These two types are often phenotypically indistinguishable and were lumped in classification.

indistinguishable from each other. Neither in the repulsion nor in the coupling cross do the ratios depart significantly from the 1:2:1 that is expected on the basis of independent assortment ($P > 0.2$ and $P > 0.7$, respectively). Summing results of the two crosses, those segregants that—were there linkage—would constitute the “non-crossover” group (single heterozygotes from the repulsion cross; agoutis and light-mottleds from the coupling cross) number 344, and the “crossover” segregants number 365. It may therefore be concluded that *Sl* assort independently of *W*^{*}.

The data in Table IV shed some light on the question of whether the type *W*^{*}/*W*^{*}; *Sl*/+ is viable. No new phenotype was found among the offspring of the light mottled by light mottled cross and, if *W*^{*}/*W*^{*}; *Sl*/+ animals are viable, they must be among the black-eyed white segregants which cannot be tested because of sterility. On the assumption that *W*^{*}/*W*^{*}; *Sl*/+ is viable, the expected number of black-eyed whites among the 61 F₂ offspring is 15.25; if it is lethal, the expected number is 5.08. The actual number of 17 black-eyed whites would seem to indicate that *W*^{*}/*W*^{*}; *Sl*/+ was viable.

Embryology

To determine the time and, if possible, cause of death of the animals missing at birth, i.e., the presumed *Sl*/*Sl* type, uterine dissections were carried out in 59 Steel females mated to Steel males. A control group consisted of 21 Steel females mated to agouti males. Results are shown in Table V.

It should first be noted that there is no difference between the two types of mating with regard to preimplantation death: the percentage of corpora lutea not represented by implants was 12.0 percent in *Sl*/+ × *Sl*/+ and 12.1 percent in *Sl*/+ × +/+ matings. It may, therefore, be assumed that preimplantation deaths are randomly distributed among the segregating genotypes, and calculations from here on will be based on total implants rather than corpora lutea.

Abnormal embryos were of two types: 1) a relatively small number, scattered through various age groups, showing malformations of the central nervous system (see footnotes to Table V for details); 2) a large group, occurring within relatively circumscribed age limits, characterized by grossly recognizable anemia. The incidence of central nervous system abnormalities was higher in the *Sl*/+ × *Sl*/+ matings but not significantly so. For comparable age groups, i.e., days 14½-17½ inclusive, there were seven among 330 morphologically observable embryos of *Sl*/+ × *Sl*/+ matings and none among 166 embryos of *Sl*/+ × +/+ matings ($t = 1.9$). Attention will, therefore, be centered on the anemic embryos as probably including the presumed *Sl*/*Sl* genotype.

In *Sl*/+ × *Sl*/+ matings, no anemics were noted in gross observations of 42 living em-

TABLE V. Observations on embryos of *Sl*/+ × *Sl*/+ and *Sl*/+ × +/+ matings

Type of mating	Total Number of							Percentage of Implants				
	Day post-conception observed	Litters	Corpora lutea	Implants	Resorbing moles	Recently dead	Living and anemic	Living, non-anemic	In advanced resorption	Recently dead	Total dead	Alive and anemic ^a
<i>Sl</i> /+ × <i>Sl</i> /+	10½	3	31	29	3	3	0	22 + 1 ^b	10.3	10.3	20.7	0
	11½	2	18	17	7	0	0	9 + 1 ^c	41.2	0	41.2	0
	12½	2	15	11	2	0	0	8 + 1 ^d	18.2	0	18.2	0
	13½	6	53	49	11	2	3 + 3 ^e	30	22.4	4.1	26.5	12.2
	14½	11	117	102	23	4	18 + 7 ^f	50	22.5	3.9	26.5	24.5
	15½	13	127	119	18	10 + 2 ^g	11 + 4 ^h + 2 ⁱ	72	15.1	10.1	25.2	14.3
	16½	10	103	88	18	12 + 2 ^g	1	55	20.5	15.9	36.4	1.1
	17½	12	121	100	20	14 + 1 ^b	1 + 3 ⁱ	61	20.0	15.0	35.0	4.0
<i>Sl</i> /+ × +/+	14½	4	37	33	4	0	0	29	12.1	0	12.1	0
	15½	5	54	45	7	2	1 ^j	35	15.6	4.4	20.0	2.2
	16½	9	96	86	10	3	1 ^j	72	11.6	3.5	15.1	1.2
	17½	3	28	25	2	1	2 ^j	20	8.0	4.0	12.0	8.0

^aIncludes doubtfully anemic embryos.

^bCollapsed brain with scalloped edges.

^cPseudencephaly.

^dKnob on top of head, brain region narrow and convoluted.

^eOne with spina bifida; one with pseudencephaly.

^fOne with spina bifida; one with spina bifida and pseudencephaly.

^gOne with spina bifida; one with collapsed brain region.

^hClear bleb slightly to midline in cervical region.

bryos aged 10½, 11½, or 12½ days (Table V). From days 13½ to 15½ the frequency of anemic embryos is high, with a peak on day 14½ where it reaches the expected 25 percent, but by day 16½ it has dropped to the level of the controls. (Anemic embryos in control, $Sl/+ \times +/+$ matings will be discussed below.) Anemia is recognized not only by the over-all paleness of the embryo but, more reliably, by observation of the blood flowing through the umbilical vessels. In normal embryos, these vessels are uniformly red; but in affected animals, it is possible to see individual clumps of red cells (or perhaps even individual cells?) moving in the bloodstream. The anemia appears to increase in severity with age of the affected embryo. Measurements of crown-rump lengths at 14½ and 15½ days showed that the anemic animals did not have retarded growth (Table VI).

In the four age groups of the $Sl/+ \times +/+$ control matings, recently dead embryos average 3.2 percent of the implants. It is clear that, in $Sl/+ \times Sl/+$ matings, the "recently dead" group is markedly increased beginning at least with day 15½. The sum of anemic plus recently dead embryos (subtracting, in each case, the appropriate control figure) is as follows: about 13 percent on day 13½, which indicates that not all of the anemias have become grossly recognizable by that time; about 25 percent on day 14½, by which time presumably all Sl/Sl embryos have expressed their anemia but few, if any, have died as yet; about 19 percent on day 15½, by which time a certain amount of death of anemics has already occurred; and 13 percent and 12 percent on days 16½ and 17½, respectively, when all, or virtually all, Sl/Sl anemics are presumably dead. The results for days 16½ and 17½ are far short of the expected 25 percent. This may be remedied by using total deaths, rather than recent deaths alone, in the calculations, i.e., by assuming that there is very rapid resorption of dead anemic embryos. This device, however, leaves very much to be desired. In the first place, it is difficult, on *a priori* grounds, to conceive of such rapid resorption so late in pregnancy; so rapid, in fact, that the change in classification from "recently dead" to "resorbing mole" would have to be achieved in one day. Secondly, the use of total rather than recent deaths raises new difficulties in the results of somewhat earlier stages. The mode of death and resorption of Sl/Sl embryos will therefore have to be further clarified by additional experiments.

TABLE VI. Crown-Rump lengths of embryos from $Sl/+ \times Sl/+$ matings*

Days post-conception observed	Non-anemic embryos		Anemic embryos	
	No. of embryos	Av. length (mm.)	No. of embryos	Ave. length (mm.)
14½	29	11.3 ± .21	17	11.0 ± .42
15½	61	13.9 ± .25	13	13.7 ± .54

*All embryos were alive at the time of measurement.

The "probably anemic" animals in the $Sl/+ \times +/+$ control matings (marked "?") in Table V) were assumed to be $Sl/+$. To test this assumption at least partially, litters from $Sl/+ \times +/+$ as well as $Sl/+ \times Sl/+$ matings were observed within a few hours after birth. It was possible to discover several young which were definitely paler than their littermates. These were marked at the time and when tested later all proved to be $Sl/+$. Determinations of this type were not done on a large enough scale to establish whether grossly anemic $Sl/+$ young are more frequent at birth than *in utero*, as seems indicated by the increase in frequency of $Sl/+$ anemics with fetal age. In other litters (which had not been observed for the presence of pale newborns), a few exploratory blood counts were made of $Sl/+$ and $+/+$ littermates after the age at which classification by coat becomes possible (Table VII). It may be seen that in four comparisons at 7-13 days of age the red counts are 20-30 percent lower in Steels than in agouti littermates. (White counts are inconsistent in the present data.) It may, therefore, be concluded that Steel heterozygotes are slightly anemic, at least until 13 days of age, and that, occasionally, this anemia may be severe enough to be detected grossly as early as 15½ days postconception. It would be of interest to determine: 1) whether the variation among $Sl/+$ animals is only in the time of onset of their anemia or also in the degree of anemia finally reached; and 2) whether the anemia is of a transitory nature. At any rate, even those $Sl/+$ animals which are anemic enough to be grossly diagnosed at birth are apparently viable.

Discussion

Even a casual comparison reveals similarities between Sl and the mutants at the *w*-locus^{1,8}: in both cases the effects are on coat pigment as well as on the blood picture. In their effects on the coat, Sl and W^s resemble each other even further in that each of them, in heterozygous condition, produces spotting as well as general slight dilution of color. The fact that, in the double heterozygote, $Sl/+; W^s/+$, the two genes appear to interact with respect to both spotting and dilution would seem to indicate that they affect similar processes.

Both Sl and W^s are semi-dominant with respect to the anemia. While $Sl/+$ and $W^s/+$ are apparently equally viable, Sl/Sl animals are much more severely affected than W^s/W^s

TABLE VII. Blood counts in $Sl/+$ young and $+/+$ littermates

Age in days	$+/+$		$Sl/+$	
	White	Red	White	Red
7	5,140	4,485,000	5,540	3,550,000
7	5,720	4,455,000	5,680	3,125,000
8	4,200	6,480,000	4,980	5,250,000
13	7,640	6,260,000	4,240	4,465,000

and even more severely than W/W , whose death is entirely postnatal. The comparison of the anemias cannot be further pursued at this time, since no detailed haematological studies of Steel homozygotes or heterozygotes have been made to date.

In Sl/Sl embryos, anemia is first grossly recognized at 13½ days, and has presumably become expressed in every Sl/Sl by 14½ days. The liver starts acting as a haemopoietic organ on the 13th day of embryonic life.¹ If it can be assumed that gross manifestation of anemia gives at least a fair indication of the real time of onset of the haematological disorder, then it may be tentatively concluded that the disturbance in Sl/Sl embryos is in some way associated with liver haemopoiesis and it may be predicted that abnormalities of the intermediate rather than the primitive blood cells will be found in anemic Sl/Sl embryos. Whatever the nature of the disturbance, it is evidently of a more severe nature than in flexed tail, f .^{1,2} In f/f animals there is a recovery from anemia when the intermediate cells are replaced by definitive red cells, but Sl/Sl embryos die before the bone marrow even starts its haemopoietic function. It will be of interest to study $Sl/+$ heterozygotes with a view to determining whether their anemia is transitory.

Summary

A dominant mutation, Steel (Sl) arose in the C3H strain. Heterozygotes are characterized by slight dilution of fur color, light ears, feet, tail and vibrissae, and a number of small white spots (tip of snout, forehead, belly) whose occurrence depends somewhat on genetic background. Homozygotes die prenatally, probably on days 15-16 postconception. They are characterized by a severe anemia which is first grossly recognizable on day 13½. Heterozygotes have a reduced red cell count but are viable.

Sl is not an allele of W^* , but the two genes interact in their effects on the coat. Sl and W^* assort independently.

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MULTIPLE NEUROFIBROMATOSIS

(Continued from page 122)

used to mean different things at different times and abused to the point where they can only cause confusion to many an eager geneticist or physician. Surely, some well-defined, functional terms can be agreed upon.

The authors estimate the frequency of neurofibromatosis from the Michigan data at one in 2,500 to 3,300 births. The fertility of persons affected with the disease, calculated by the Krooth method, is found to be surprisingly low, with that of the males reduced to less than 50 percent.

Multiple neurofibromatosis has long been known to behave as a dominant trait. From analysis of their familial cases the authors find "no evidence that the dominant gene responsible for neurofibromatosis does not enjoy a high level of penetrance." The sporadic cases are assumed to represent new mutations and for some cases the authors present evidence that this is so.

The authors also attempt to calculate a mutation rate for this gene from their material. This, they estimate at 1×10^{-4} , which admittedly is the highest rate for a dominant gene known. The pitfalls and biases involved

in the calculation of a mutation rate are numerous and difficult to control and the authors are the first to admit it. In the present case, the mutation rate was estimated under the assumption that the gene had complete penetrance. Yet, previous studies have shown it to skip generations occasionally or favor one sex over the other. Not the least of the biases which may raise the estimate of a mutation rate is the failure to investigate extensively the family histories of the propositi branching into second and third degree relationships.

In general, the material is well presented and the conclusions well documented. As an attempt at integrating the disciplines involved, the book is not altogether successful, but it is a worthy effort and we hope that other monographs of this design will soon follow.

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