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Mi-spotted: a mutation in the mouse*

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A cross between a C57BL/6J ♀ and C57BL/6J-Mi^{wh}/+♂ produced five deviant mice. These mice were pale yellow and had well-demarcated white spots both on the dorsum and ventrum (that on the ventrum was one large spot). They had pigmented eyes, but tail, feet, and ears were largely unpigmented. Hairs that were initially pale yellow lost their yellow appearance at first moult and became 'sooty'.

The parental cross in which the deviants occurred was from backcross generation 44 in stock C57BL/6J-Mi^{wh}, where Mi^{wh} (white) is maintained on the C57BL/6J genetic background by repeated backcrossing. Coat colour phenotypes from the parental cross were expected to be either grey (Mi^{wh}/+) or black (+/+) in a 1:1 ratio. The occurrence of deviants by litter is given in Table 1.

Table 1. *Distribution of deviants from a C57BL/6J ♀ × C57BL/6J-Mi^{wh}/+♂ cross*

		Coat colours classified					
		Black		Grey		Yellow-spotted	
Litter	No. born	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	5	1	2	0	1	0	1
2	3	1	2	0	0	0	0
3	9	1	2	2	0	3	1
Totals	17	3	6	2	1	3	2

1. GENETIC TESTS

To test if the mutation was genetic and to determine its mode of inheritance, the first male deviant occurring in litter 1 (see Table 1) was crossed to three C57BL/6J ♀♀ that were not closely related to the parental C57BL/6J ♀. The four deviants from litter 3, apparently identical to the first deviant, were intercrossed. Results from these crosses, together with intercrosses of their progeny, are given in Table 2. All crosses were made within C57BL/6, i.e. either strain C57BL/6J or C57BL/6J-Mi^{wh},

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and descriptions of coat colour consequently are for a non-agouti (a/a) background. Mice were classified at weaning (30 days).

It is evident from the data of Table 2 that a mutation has occurred at (or very near) the microphthalmia (*mi*) locus in linkage group XI. This new mutation was earlier named *mi*-spotted (mi^{sp}) (Wolfe, 1962), which is in accord with rules established by the Committee on Standardized Genetic Nomenclature for Mice (1963).

Table 2. *Progeny from crosses and intercrosses of mutant mice classified at weaning. All mice were* a/a*

Cross	Matings	Observed: Grey	Black	Yellow-spot	White	Total	Expected ratio	X^2
I. Yellow-spot × C57BL/6J ($Mi^{wh}/mi^{sp} \times +/+$)	3	41	47			88	1:1	0·14
Intercrosses from I:								
Ia. Grey × Grey ($Mi^{wh}/+ \times Mi^{wh}/+$)	2	11	7		9	27	1:2:1	0·69
Ib. Grey × Black ($Mi^{wh}/+ \times mi^{sp}/+$)	7	31	60	30		121	1:2:1	0·02
Ic. Black × Black ($mi^{sp}/+ \times mi^{sp}/+$)	3		73			73		
II. Yellow-spot × Yellow-spot ($Mi^{wh}/mi^{sp} \times Mi^{wh}/mi^{sp}$)	3		13	30	16	59	1:2:1	0·32
Intercrosses from II:								
IIa. Black × Black ($mi^{sp}/mi^{sp} \times mi^{sp}/mi^{sp}$)	3		64			64		
IIb. Black × White ($mi^{sp}/mi^{sp} \times Mi^{wh}/Mi^{wh}$)	2			14		14		
IIc. Yellow-spot × White ($Mi^{wh}/mi^{sp} \times Mi^{wh}/Mi^{wh}$)	2			27	24	51	1:1	0·09
IId. Yellow-spot × Black ($Mi^{wh}/mi^{sp} \times mi^{sp}/mi^{sp}$)	2		7	15		22	1:1	1·45

* Hypothesized genotypes are shown in parentheses.

The coat colours of mice that correspond to genotypes given in Table 2 are as follows: Grey = $Mi^{wh}/+$; black = $+/+$, $mi^{sp}/+$, or mi^{sp}/mi^{sp}; yellow-spot = $Mi/^{wh}$ mi^{sp}; and white = Mi^{wh}/Mi^{wh}. A critical test of the hypothesis is Cross IIb of Table 2; in crosses of $mi^{sp}/mi^{sp} \times Mi^{wh}/Mi^{wh}$ only yellow-spot animals were recovered as predicted. Further, mice of genotype Mi^{wh}/mi^{sp} transmit either Mi^{wh} or mi^{sp} but not both in the same gamete. *Mi*-spotted is neither dominant nor recessive in the usual sense, i.e. in crosses of mutants to C57BL/6J (Cross I) no mutant types were recovered. This seemed at first to indicate typical recessive transmission, but in intercrosses between putative mi^{sp}/mi^{sp} homozygotes (Cross IIa), only wild-type offspring were recovered. Thus mi^{sp} expresses itself visibly only when in allelic

combination with Mi^{wh}, and yellow-spot mice are of genotype Mi^{wh}/mi^{sp}. No visible differences between $+/+$, $mi^{sp}/+$, and mi^{sp}/mi^{sp} are evident. There is also no evident viability or fertility effect associated with mi^{sp}, since litter size and proportion of fertile matings were normal for the different crosses. Strain C57BL/6J mice that were closely related in the pedigree to the parent C57BL/6J female producing the mutants, produced no mutant offspring when mated to Mi^{wh}. Since mi^{sp} does not confer any obvious selective advantage, it is reasonable to assume that the mutation arose spontaneously in the parent C57BL/6J female or its immediate ancestors, and is absent in the currently existing C57BL/6J strain.

Crosses were subsequently made with mice heterozygous for microphthalmia (*mi*), imported to The Jackson Laboratory through the courtesy of Dr Hans Grüneberg. Crosses of *mi*, mi^{sp} and Mi^{wh} in all possible combinations gave predicted classes and reaffirmed the location of mi^{sp} at the *mi* locus. Animals of genotype mi/mi^{sp} were all-white except for a variable number of faint irregular patches of pigment, though eye pigmentation was reduced over that of $mi/+$; eye pigmentation was nearly indistinguishable from that of C57BL/6J-*ru*/*ru* (ruby) mice.

Microphthalmia is being backcrossed onto the C57BL/6J background and is at generation 8 at this writing. The faint dorsal pigmentation of mi/mi^{sp} animals present in early crosses has not been observed in animals of this same genotype subsequent to generation 4, all hair being devoid of pigment.

2. ASSAYS OF TYROSINASE ACTIVITY IN THE SKIN

Though $mi/^{sp}+$ and mi^{sp}/mi^{sp} are indistinguishable from wild-type ($+/+$) on gross examination, tyrosinase activity in skin slices among these genotypes differ. Tyrosinase activity was determined by one of us (D.L.C.) by measuring the amount of tyrosine-2-C^{14} incorporated into the melanin of the skin of 5-day-old animals per unit of dry weight following a method previously described (Coleman, 1962). The assay was extended to include genotypes $mi/+$, $Mi^{wh}/+$, Mi^{wh}/mi^{sp} and Mi^{wh}/Mi^{wh}. All mice examined were highly congenic with C57BL/6J, the genes at the microphthalmia locus either having been backcrossed to C57BL/6J for eight or more generations (*mi* and Mi^{wh}) or having originated in C57BL/6J (mi^{sp}). Results are given in Fig. 1.

Among those mice which were phenotypically black, $+/+$ showed most activity followed in turn by $mi^{sp}/+$, $mi/+$ and mi^{sp}/mi^{sp}. Mice of genotype $mi^{sp}/+$ differ significantly from mi^{sp}/mi^{sp} ($P < 0{\cdot}01$) and both differ significantly from wild-type ($+/+$) ($P < 0{\cdot}05$). Mice of genotype $mi/+$ do not differ from either $mi^{sp}/+$ or mi^{sp}/mi^{sp} but differ from all other genotypes examined. Skins of genotype $Mi^{wh}/+$ incorporated a significantly greater amount of tyrosine than those of the yellow mutant Mi^{wh}/mi^{sp} ($P < 0{\cdot}001$) though there is some doubt as to the validity of this comparison. In some cases the 1×2 cm. section of dorsal skin that was assayed overlapped some faintly visible white spots and this appeared to reduce the count. Counts from Mi^{wh}/mi^{sp} were about equal to those from $Mi^{wh}/+$ in three samples where white spotted areas were deliberately avoided. Tyrosine incorporation was low in mice of the all-white genotype Mi^{wh}/Mi^{wh}. Excepting those genotypes that

were phenotypically black, the amount of tyrosine incorporated was directly proportional to the degree of hair pigmentation possessed by the different mutant types.

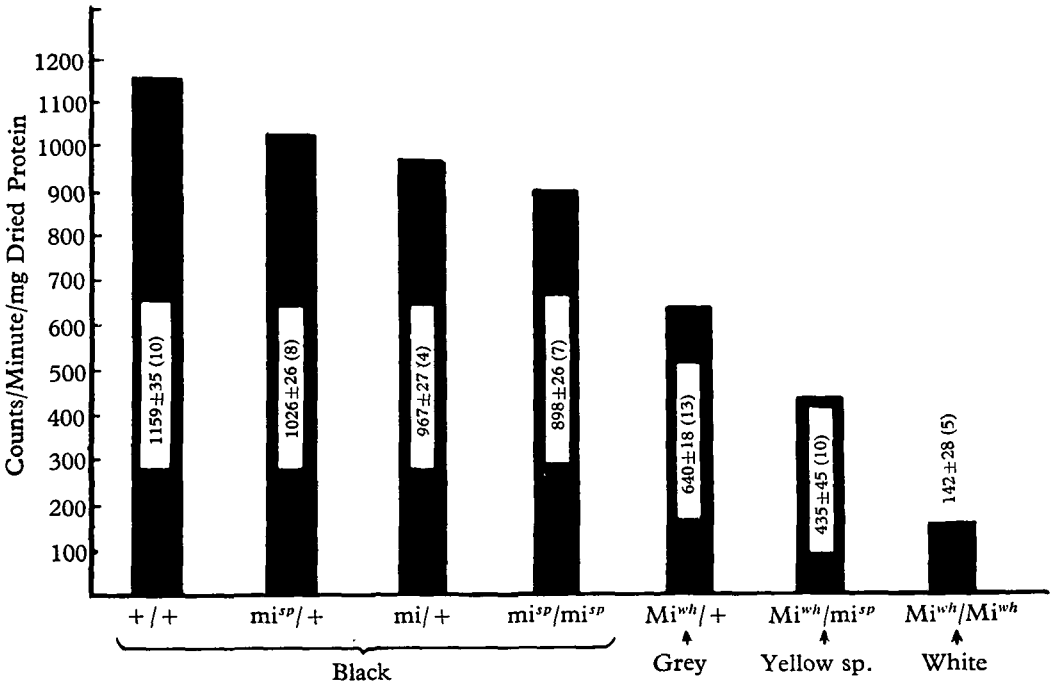

Fig. 1. Tyrosine-2-C^{14} incorporation in skin slices of 5-day-old mice differing at the microphthalmia locus. Means, standard errors, and number of determinations are shown for each genotype. All mice were *a*/*a*.

3. PIGMENT GRANULES IN THE HAIR

Since the lowered enzyme level in $mi^{sp}/+$ and mi^{sp}/mi^{sp} types might be related either to number, kind, or distribution of pigment granules in the hair shaft, or to attributes of the skin only, it was important to examine the hairs of the different genotypes. Club hairs from the first pelage (4- to 5-week-old weanling mice) were mounted on slides by the method of Russell (1946). A survey of different hair types revealed no important differences in pigmentation that might be associated with hair types. This observation agrees with that reported by Russell (1946). Hence, zigzags, the most numerous type, were selected for detailed studies as representative of the whole coat. Whole mounts were prepared from five mice of each of six non-agouti genotypes as given in Table 3. Both cortical and medullary granules were enumerated in three zigzag hairs of each preparation at four locations along the hair shaft, at points equidistant between apex and first constriction, or between constrictions. (Zigzag hairs of the mouse usually have three constrictions (Dry, 1926).) For convenience, these regions will be referred to as (beginning from apex) Regions I, II, III, and IV. At the selected points, the hair shaft is usually of greatest diameter, and generally this is the region of greatest medullary pigmentation for that particular

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section of the hair, though not usually the area of greatest cortical pigmentation. Cortical pigment is heaviest near the tip of the hair and falls off rapidly toward the base. An excellent account of cortical and medullary granulation in thirty-six different genotypes, not including the *mi* locus, is given by Russell (1946). Medullary granules were counted for one cell and cortical granules were counted over a single interseptal distance, which varied from 7 to 10 μ in length.

Table 3. *Effects of genic substitution at the microphthalmia locus on number and distribution of cortical and medullary pigment granules in the hair shaft. See text for a definition of regions of the zigzag hair. Number of granules in each instance are means of* 15 *samples. All mice were* a/a

	$+/+$	$mi^{sp}+$	$mi/+$	mi^{sp}/mi^{sp}	$Mi^{wh}/+$	$Mi/^{wh}mi^{sp}$
Cortex						
First 50μ from apex	26 ± 6	30 ± 7	20 ± 4	27 ± 3	0	0
Region I	*	*	*	*	42 ± 5	0
Region II	42 ± 8	47 ± 7	44 ± 6	37 ± 5	26 ± 6	$0{\cdot}5 \pm 0{\cdot}2$
Region III	7 ± 1	6 ± 1	6 ± 1	5 ± 1	12 ± 3	0
Region IV	2 ± 1	3 ± 1	2 ± 1	3 ± 1	4 ± 1	0
Medulla						
Region I	*	*	*	*	30 ± 2	3 ± 1
Region II	*	*	*	*	63 ± 4	16 ± 4
Region III	*	*	*	*	70 ± 4	15 ± 3
Region IV	*	*	*	*	54 ± 3	11 ± 2

* Too dense for accurate counting.

Results are given in Table 3. No differences were observed in number, kind or arrangement of cortical granules among $+/+$, $mi^{sp}/+$, $mi/+$ or mi^{sp}/mi^{sp} genotypes. Nor were there any differences in distance to first cortical pigment measured from the apex of hairs. These were $+/+$, $26 \pm 2\,\mu$; $mi^{sp}/+$, $25 \pm 2\,\mu$; $mi/+$, $27 \pm 3\,\mu$; and mi^{sp}/mi^{sp}, $24 \pm 3\,\mu$. Medullary granules were not enumerated in these genotypes; granules were compactly grouped and could not be counted without a large error. The granules did not appear to differ from those of wild-type. The latter (*aa BB CC DD PP*) have been characterized by Russell (1946) as long oval and intense black from her studies of cross-sections. There were no observed pigment granules in Mi^{wh}/Mi^{wh}, mi/mi^{sp}, and mi/mi hairs.

$Mi^{wh}/+$ exhibits what Russell (1946) has called 'pigmentation lag'. There is an apparent delay in deposition of medullary pigment in the growing hair. Pigmentation lag is even more pronounced in Mi^{wh}/mi^{sp}. Peak medullary granulation in $Mi^{wh}/+$ and Mi^{wh}/mi^{sp} is not reached until Region II–III (see Table 3). First cortical granules observed, as measured from the apex of the hair shaft, were $Mi^{wh}/+$, $150 \pm 20\,\mu$, and Mi^{wh}/mi^{sp}, $1567 \pm 220\,\mu$. In some hairs of Mi^{wh}/mi^{sp} there were no cortical granules along the entire length. Consequently most pigmentation of the

coat in these genotypes comes from granules located in the proximal portion of the hair shaft.

Medullary granules of $Mi^{wh}/+$ and particularly Mi^{wh}/mi^{sp} vary greatly in size and shape. These vary from 0·4 μ to as much as 5 μ in greatest diameter in Mi^{wh}/mi^{sp} and appear to be a result of clumping. Granules appear less dense than wild-type and have a yellowish appearance when viewed by transmitted white light. There is less variation in size and shape of granules in post juvenile hairs, and there are more cortical and medullary pigment granules, in accord with earlier observations of Grobman & Charles (1947) on hairs of $Mi^{wh}/+$ mice.

4. DISCUSSION

From the breeding data presented, it is apparent that mi^{sp} is a new allele at the *mi* locus. The manner of its discovery is unusual. Since it has no visible effect in either single or double gene dose, mi^{sp} was detected only by its interaction with Mi^{wh}. In this regard mi^{sp}–Mi^{wh} might be likened superficially to the *t-T* (tailless-Brachyury) relationship. The *t* alleles all produce a tailless phenotype when combined with *T* and are first detected in this way. Differences among them have been detected by other, less obvious properties (see Dunn *et al.*, 1962, for a recent tabulation of alleles).

Though no obvious effect on hair pigmentation by mi^{sp} is evident in the absence of mutant alleles at the *mi* locus, differences are readily apparent in tyrosinase activity in the skin of 5-day-old mice. The differences in tyrosinase activity do not cause differing amounts of pigment to be deposited in the hair, however. In number and kind of pigment granules, the hairs of $mi^{sp}/+$ and mi^{sp}/mi^{sp} are indistinguishable from each other and from $+/+$. Possibly, as suggested by Coleman (1962) for alleles at the albino (*c*) locus, there is a threshold level for tyrosine incorporation, above which full pigmentation can occur. Our data suggest that this threshold falls between 640 and 898 c.p.m. compared to 600 c.p.m. for the *c* locus series.

There need not be a correspondence between tyrosinase activity in the skin and volume of pigment in the hair. Coleman (1962) found that tyrosine-2-C^{14} incorporation in skins of mice of *a/a; b/b* genotype was approximately twice that of mice of *a/a; B/B* genotype (2680, cf. 1200 c.p.m.), though total pigment volume in the hair of a/a; b/b is less (Russell, 1948). The reason for greater tyrosine incorporation in *a/a; b/b* may be a greater number of tyrosinase binding sites on the matrix protein of the developing melanosome (Moyer, 1964).

Tyrosine incorporation in Mi^{wh}/mi^{sp} was less than in $Mi^{wh}/+$ and this corresponds with a reduction in amount of pigment in the hair. There is apparently some tyrosinase activity even in Mi^{wh}/Mi^{wh} (but no granules in the hair) since counts were greater than those obtained by Coleman (1962) for albino (*c/c*) and extreme dilution (c^e/c^e) where tyrosinase is known to be absent or at very low concentration. Tyrosine incorporation was less in $mi/+$ than in $+/+$, yet hair pigment was not reduced. With regard to the latter comparison, it has long been known that $mi/+$ heterozygotes can be visibly distinguished from $+/+$, particularly on a *A/A; b/b* background, by a reduction of pigment in the choroid and iris of the eye (Grüneberg, 1952).

Mi^{wh} in single gene dose causes dilution of the fur and a characteristic belly spot on the C57BL/6J genetic background. Choroid and harderian gland of a/a; $Mi^{wh}/+$ or a^t-a^t $Mi^{wh}/+$ are not pigmented (i.e. spotted) though melanocytes function in ear skin, hair follicles, and nictitans (Markert & Silver, 1956). The allele mi^{sp} modifies (increases) the spotting of Mi^{wh} as evident in the compound a/a; Mi^{wh}/mi^{sp}. The dorsal spotting pattern is similar to that found in some piebald spotting. Black-eyed white (mi^{bw}) has an effect similar to mi^{sp} when in combination with Mi^{wh} on a non-agouti (a/a) background (Schaible, 1963). However, pale yellow areas are perhaps more dilute and more restricted in size in the latter. Nothing is known of mi^{sp}–mi^{bw} interaction.

Prospective pigment cells (or melanoblasts) of the mouse, except those of the retinal pigment epithelium which come from the outer wall of the optic cup, arise from cells of the neural crest (Rawles, 1947). These cells migrate to different tissues including the receiving hair follicle, where they differentiate into mature melanocytes. A mutant gene causing restriction of pigment (spotting) may potentially act at any stage, from differentiation of the melanoblast from neural crest to the time of its final differentiation to melanocyte within the hair follicle. The important alternatives to distinguish, and they need not be mutally exclusive, is whether gene action is mediated via the cellular environment or resides within the melanoblast.

Markert & Silvers (1956) inferred that presumptive melanoblasts do not differentiate at their origin in the neural crest, are prevented in some way from migrating, or do not differentiate once they have reached their definitive positions in all-white mice of genotype Mi^{wh}/Mi^{wh}. It is convenient to think of these mice as having 'one big spot' (Silvers, 1956). All-white mice of genotypes Mi^{wh}/mi, mi/mi, mi/mi^{sp} and mi^{bw}/mi^{bw} (black-eyed white; see Kreitner, 1957; Strong & Hollander, 1963) probably lack skin and hair pigments for similar reasons. Markert and Silvers explanted mi^{bw}/mi^{bw} embryonic tissue containing neural crest into the anterior chamber of the eyes of albino hosts. No pigment cells were ever obtained from these grafts (Markert, 1960), a result indicative of failure of neural crest cells to differentiate into melanoblasts. Evidence that melanoblasts never reach a near-functional state in the receiving hair follicle is the absence of clear cells (amelanotic melanocytes) such as found in hair follicles of albino mice, in hair follicles of white spotted areas (Silvers, 1953, 1956).

It is not clear how the failure of cell differentiation in Mi^{wh}/Mi^{wh} relates to the dilution effect in $Mi^{wh}/+$. It is not a simple reduction in number of melanoblasts and consequently of quantity of hair pigment in pigmented areas of $Mi^{wh}/+$. 'Pigmentation lag' and presence of small granules in the hair suggests that an unfavourable follicular environment may interfere with normal deposition of granules, or that melanoblasts have differentiated into cells incapable of synthesizing normal pigment granules. The latter explanation is likely since Silvers & Russell (1955) showed that neither skin nor hair follicle of Mi^{wh}/Mi^{wh} inhibited melanogenesis. They transplanted foetal or neonatal skin of a/a; Mi^{wh}/Mi^{wh} onto neonatal mice of genotypes a/a; C/C and A^y/a; C/c^e (ventrum to dorsum). Melanoblasts invading the a/a; Mi^{wh}/Mi^{wh} graft produced black pigment.

Pigment granules in hairs of mice of genotype Mi^{wh}/mi^{sp} are yellow-brown when viewed by transmitted white light and appear less dense than those of $Mi^{wh}/+$ and wild-type. The morphology of Mi^{wh}/mi^{sp} granules is basically different from that of the yellow granules produced by alleles at the agouti locus. Whereas Mi^{wh}/mi^{sp} granules vary from small to large and are of irregular shape, yellow granules in hairs of A^y/a mice are small and round, and show little variation among genotypes differing at four other pigment-affecting loci (Russell, 1946).

In a series of skin grafting experiments, Silvers & Russell (1955) demonstrated that genic expression of alleles at the agouti (A) locus depends on the genotype of the follicular environment and the location of this environment on the integument of the mouse. Cleffmann (1963) found that melanocytes of dorsal hair follicles from skin explants of unborn and young mice differing at the agouti locus, including black and tan (a^t) and non-agouti (a) alleles, could be forced to form yellow pigment by addition of glutathione or other sulph-hydryls to the culture medium. Though present evidence for the *mi* locus, unlike that for the A locus, favours failure of melanoblast differentiation and does not exclude some genic control within the melanoblast, it would nevertheless be instructive to observe the kind of pigment produced by invasive mi^{sp}/mi^{sp} melanocytes in a Mi^{wh}/Mi^{wh} skin graft. Studies of the fine structure of melanocytes of $Mi^{wh}/+$ and Mi^{wh}/mi^{sp} genotypes, such as those by Moyer (1964) on retinal pigment epithelium of mutant mice, might reveal differences in melanosome maturation.

SUMMARY

Mi-spotted (mi^{sp}) is a new mutation in the mouse at the microphthalmia (*mi*) locus. It has no obvious visible effect in either the heterozygote ($mi^{sp}/+$) or homozygote (mi^{sp}/mi^{sp}) and was discovered by virtue of its interaction with white (Mi^{wh}). Mice of genotype Mi^{wh}/mi^{sp} are pale yellow with white spots; yellow areas later become 'sooty' at the first moult.

Though mice of genotypes a/a; $mi^{sp}/+$ and a/a; mi^{sp}/mi^{sp} cannot be visibly distinguished from those of a/a; $+/+$, the amount of tyrosine-2-C^{14} incorporated in melanin in skins of 5-day-old mice of these genotypes differed. Assays of tyrosine incorporation were extended to include other non-agouti genotypes differing only at the microphthalmia locus. The amount of tyrosine incorporated was greatest in $+/+$ followed in order by $mi^{sp}/+$, $mi/+$, mi^{sp}/mi^{sp}, $Mi^{wh}/+$, Mi^{wh}/mi^{sp}, and Mi^{wh}/Mi^{wh}.

Pigment granules were examined in club hairs of these same genotypes for different regions of the hair shaft. Hairs of $mi^{sp}/+$, mi^{sp}/mi^{sp}, and $mi/+$ could not be distinguished from $+/+$ either in number, kind or arrangement of granules. Hairs of $Mi^{wh}/+$ showed reduced cortical and medullary pigment, especially distally, which was even more pronounced in hairs of Mi^{wh}/mi^{sp}. Medullary granules of Mi^{wh}/mi^{sp} varied greatly in size with a few large yellow-brown granules.

The new mutants described were found in the colour stocks of mice maintained by Dr Elizabeth S. Russell. We wish to thank her for making space available for the breeding experiments and for valuable criticisms. We also wish to thank Miss Edith Kent, Miss Judith Ellis and Mr Richard Copp for their technical assistance.

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