

SOME OBSERVATIONS ON THE MICROPHTHALMIA GENE IN THE MOUSE

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(With Six Text-figures)

INTRODUCTION

In May 1946 I obtained through the good offices of Prof. R. A. Fisher (Cambridge) three mice heterozygous for the microphthalmia gene described by Paula Hertwig (1942*a, b*) and derived from her colony. On the offspring of these animals certain observations have been made which will be reported in this paper. Contact subsequently established with Prof. Hertwig (Halle/Saale, Germany) revealed the fact that she herself had continued studies on the mutant throughout the war and that her unpublished material includes at least one of the observations independently made by the present author. To avoid further duplications, work on the strain has been discontinued in this laboratory (except as mentioned below) and the new data are presented here, incomplete and disconnected though they are.

THE MICROPHTHALMIC HOMOZYGOTE

The microphthalmic homozygote, as described by Hertwig (1942*a, b*), is one of the most striking cases of manifold gene effects (pleiotropism) known in mammals. There is a complete absence of all pigment from fur and eyes, and in this respect the animal is indistinguishable from an ordinary albino mouse. As the name of the mutant implies, the eyes are reduced in size and do not show between the closed eyelids. According to Hertwig, the reduction of eye size is regularly associated with a coloboma of the retina. The tips of the vibrissae (whiskers) tend to be bent. Except in rare instances, the incisors are retained in the jaws and do not erupt. Nearly all the animals die on weaning, but Hertwig observed one male which reached sexual maturity and bred; mated to an ordinary albino female it produced one coloured young and thereby proved the two genes to be different from each other. Hertwig's use of the symbol *m* needs revision, as that symbol had previously been used for the gene misty. The symbol *mi* will be used in this paper.

The only other gene in the mouse which causes retention of teeth is the grey-lethal gene (Grüneberg, 1935-8) which also eliminates the yellow pigment, though the black pigment is unaffected. In the case of the grey-lethal (*gl/gl*) mouse, the retention of the teeth is a consequence of a complete lack of secondary bone absorption which leads to characteristic bone deformities throughout the whole skeleton.

A comparison of skeleton and teeth of the grey-lethal and the microphthalmic mouse showed an extraordinary degree of resemblance. As evidenced by Figs. 1-3, the various bones are almost photographically alike and, in either case, show all the peculiarities of shape which follow from a lack of secondary bone remodelling. Histologically the bones of the microphthalmic mouse show precisely the same persistence of spicules throughout the marrow cavities which is so characteristic of the grey-lethal mouse. The only major differences between the two mutant skeletons so far discovered occur on the mandible and

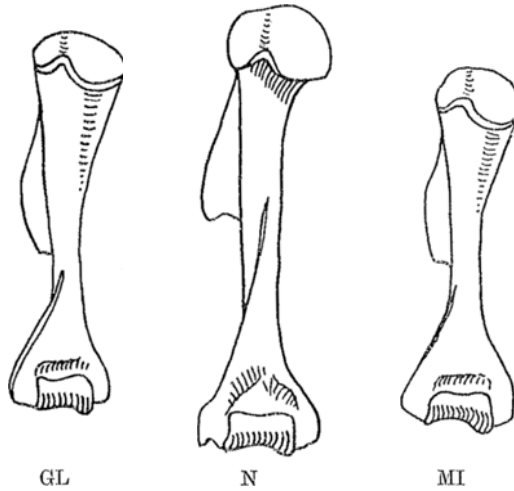


Fig. 1. Humeri of a grey-lethal (GL), a normal (N) and a microphthalmic mouse (MI), 17 days old. Note in both abnormal animals particularly the conical shape of the proximal end of the bone with absence of a collum chirurgicum; the abnormal extension distally of the crista deltoidea; and the abnormal configuration of the fossa olecrani. Camera lucida drawings.

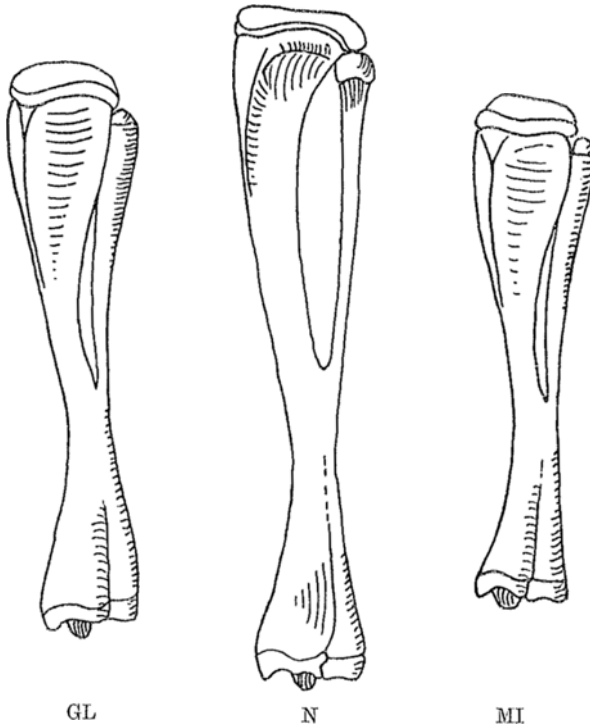


Fig. 2. Tibiae and fibulae of the same animals as in Fig. 1. Note the lack of tubulation particularly at the growing (proximal) ends of both bones in both the grey-lethal and the microphthalmic mouse.

upper jaw. In the grey-lethal the rear end of the lower incisor invariably erupts backwards through the foramen mentale which is greatly dilated. In the microphthalmic mouse this happens in some individuals, but not in others. This is as one might expect in view of the fact that the incisors occasionally erupt in a normal fashion. While this is very exceptional, a delayed and incomplete eruption of molars, either in the mandible or in the upper jaw, or in both, is quite a common occurrence in microphthalmic mice, and during the fourth week of life the majority of the animals show signs of eruption of some of the molar teeth. The second molars are more often successful than the first ones, but occasionally a complete or almost complete eruption of all the first and second molars occurs. Whether the third molars can erupt is uncertain, as no animals old enough to show this have so far been dissected. The frequent eruption of molars and the occasional eruption of incisors clearly demonstrate that in the microphthalmic mouse secondary bone absorption, though greatly reduced, is not altogether absent. Evidently the stimulus

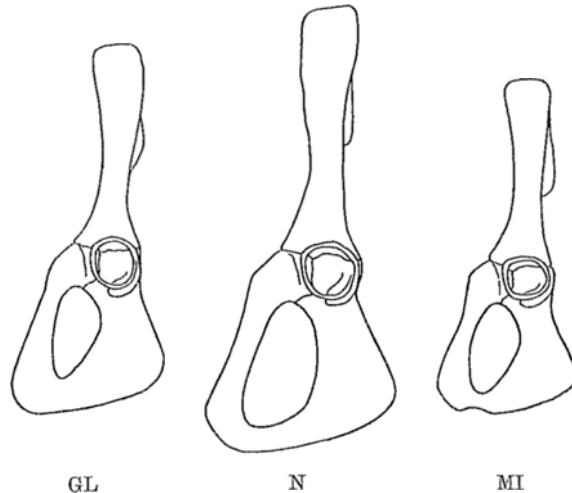


Fig. 3. Pelves of the same animals as in Figs. 1 and 2. Note particularly the abnormal shape of the foramen obturatum in both abnormal animals.

of the erupting teeth is in this case often sufficient to induce absorption of the overlying alveolar bone which never happens in the grey-lethal mouse.

While the *erupting* tooth of the microphthalmic mouse is thus obviously a sufficient stimulus for bone absorption, this does not seem to be so in the case of the *growing* (but not yet calcified) crowns of the first molars. In the grey-lethal, characteristic shape anomalies in both upper and lower first molars develop from this fact (Grüneberg, 1937), and just the same kind of shape anomaly is found in the corresponding teeth of the microphthalmic mouse (see Fig. 4). The correspondence of both skeleton and teeth down to such minute details is thus extremely close, and but for the few differences in the jaws mentioned above the skeletons are almost indistinguishable from each other. A detailed comparison of the *gl/gl* and *mi/mi* skeletons with each other and with the normal skeleton is now being carried out in this laboratory and will be published later.

In view of the close resemblances described above, the suspicion arose that *gl* and *mi* might be allelomorphs and bear a relation to each other similar to that of chinchilla and albinism in the albino series of the rodents. To test this possibility, known heterozygotes

for either gene ($+/gl$ and $+/mi$ respectively) were mated. Altogether, eighty-six young were produced, all of them normal. As 21.5 compounds (presumably intermediate between gl and mi) would be expected amongst these eighty-six mice if the two genes were allelomorphous to each other, that hypothesis can be regarded as ruled out. This is confirmed by a mating between two F_1 mice which produced twenty-three normals, eight grey-lethals and nine microphthalmics. Whether the latter included any double recessives (mi/mi ; gl/gl) is uncertain, as that genotype, if viable, would presumably be indistinguishable from mi/mi phenotypically.

The genes gl and mi are thus not allelomorphs, but form a remarkable pair of 'mimic' genes. Whether these occupy different chromosomes cannot be decided from the data available, as the segregation obtained fits a 9 : 3 : 4 ratio (in the case of independent assortment) or a 2 : 1 : 1 ratio (in the case of complete linkage) almost equally well.

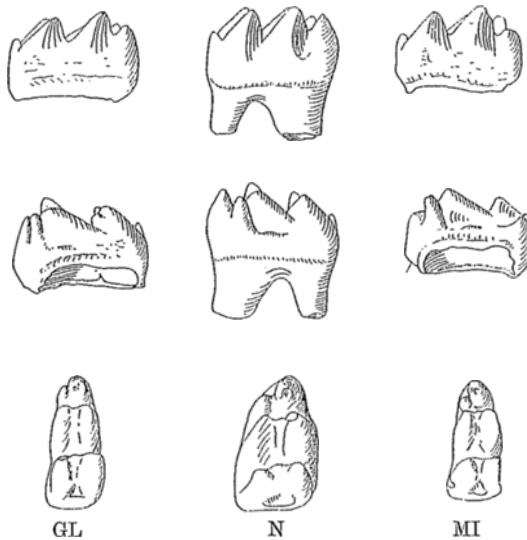


Fig. 4. Lower first molars of grey-lethal (GL), normal (N) and microphthalmic (MI) mouse, 14 days old. Note particularly the compression of both abnormal teeth in bucco-lingual direction.

THE HETEROZYGOTE FOR MICROPTHALMIA

That the gene for microphthalmia is not completely recessive has already been pointed out by Hertwig (1942*a*) who was able to distinguish newborn $+/mi$ from $+/+$ young by the reduced amount of eye pigment visible through the closed eyelids. Hertwig was unable to distinguish these two genotypes later in life 'as the eye colour is later equally dark', but mentions briefly that in the same stock of mice there occurs a second new gene for dark red eye colour to be discussed in a later communication.

The present author found no difficulty in distinguishing $+/mi$ heterozygotes from homozygous normals ($+/+$) throughout life in the various combinations with the genes for agouti *v.* non-agouti (A *v.* a), black *v.* brown (B *v.* b), and intense *v.* dilute (D *v.* d), provided the animals are inspected under strong illumination with the aid of a dissecting microscope. From a personal communication it appears that Prof. Hertwig has made the same discoveries since her first paper was published, and that she is going to publish her results in detail in due course. Here we shall give only a brief discussion of the facts sufficient to guide other workers in the identification of these genotypes.

The situation is somewhat complicated by the fact that, contrary to previous statements in the literature, the coat-colour factors a , b and d and various spotting genes affect the eye colour of the mouse quite appreciably. In normal development, the deposition of eye pigment starts as a fine ring round the pupil early on the 12th day of gestation. From this region pigment later spreads throughout the iris and the whole of the choroid; thus, by the time the young (wild-type) mouse opens its eyes the choroidal blood vessels are hidden from view by the pigment; this completely absorbs the light falling on to the retina through the pupil, and the latter appears jet black. The gene for brown dilutes the choroidal pigment, particularly in the central part of the fundus; as a consequence, cinnamon (A/A ; b/b) mice under a strong light and a dissecting microscope show a dull red reflex in the pupil in adult life and a vividly red one at the age of 2–3 weeks when the

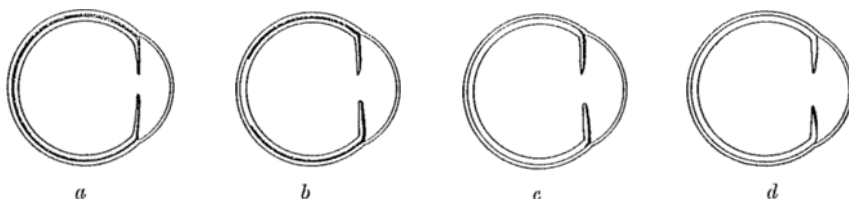


Fig. 5. Distribution of eye pigment in normal (a) and $+/mi$ mice. Explanation in the text.

deposition of pigment is not yet complete. On the other hand, the non-agouti gene (a/a) increases the amount of choroidal pigment both in black and brown animals; as a consequence non-agouti browns (chocolates) in adult life as a rule show no pupillary reflex whatsoever.

The mi gene in heterozygous condition reduces the choroidal pigment in a similar manner to brown (b/b), but much more powerfully, as shown diagrammatically in Fig. 5. The reduction starts in the centre of the fundus which is normally reached last by choroidal pigmentation (Fig. 5*b*). The next step is a reduction of the choroidal pigment gradually spreading towards the periphery of the fundus (Fig. 5*c*); this happens particularly in those genotypes in which the choroidal pigment is already reduced by the gene b , such as in chocolates and cinnamons; in such animals, if the eye is luxated from the orbit by gentle pressure of the finger, the sclera next to the limbus corneae is slightly transparent and pink, whereas in normally pigmented eyes the choroidal vessels are hidden by the dense pigment and the sclera appears opaque and slate-grey. Finally, in $+/mi$ cinnamons, the reduction often goes so far that the periphery of the iris is free of pigment and transparent, leaving some pigment only round the pupillary margin (Fig. 5*d*); the eye of such animals appears a dark red or ruby colour under ordinary illumination, and these are the mice which Hertwig (1942*a*) mentioned as due to a second gene in the stock. The situation is summarized for a few genotypes in Table 1.

Table 1. Choroidal pigmentation in adult $+/mi$ and $+/+$ mice

		ABD	AbD	aBD	abD	abd
Pupillary reflex	$+/mi$	++	+++	+	++	++
	$+/+$	-	+	-	-	(+)
Sclera transparent	$+/mi$	(+)	+++	-	++	++
	$+/+$	-	-	-	-	-
Iris transparent	$+/mi$	(+)	++	-	(+)	(+)
	$+/+$	-	-	-	-	-

In the details there may be considerable differences within each genotype, and there are sometimes striking asymmetries; for instance, in $+/mi$ cinnamons it often happens that one eye is frankly ruby in colour, while the other looks almost black on casual inspection. In wild-type (black agouti) $+/mi$ heterozygotes, the transparency of the sclera is very variable and often quite absent; on the other hand, some animals of this genotype show a reduction of iris pigmentation sufficient to make the periphery of the iris slightly transparent.

That the classification of $+/mi$ as opposed to $+/+$ by adult eye pigmentation is generally accurate can be inferred from the segregations obtained (Table 2) which do not differ significantly from the ratios expected. Furthermore, all forty-two $+/mi$ classified

Table 2. *Segregation of the mi gene. Classified by eye colour*

Type of mating	$+/+$	$+/mi$	mi/mi	Total
$+/mi \times +/+$	185	169	—	354
$+/mi \times +/mi$	259	511	270	1040

Note. $+/+$ and $+/mi$ in both types of mating were classified at the age of 3 weeks or over. The mi/mi homozygotes in the intercross were classified by the absence of eye pigment at birth; as some of the $+/+$ and $+/mi$ died before classification, the data are biased in favour of mi/mi ; the ratio at birth was 850 $+/+$ and $+/mi$: 270 mi/mi , a close fit to a 3:1 ratio.

as such from their adult eye colour and used for breeding have behaved according to expectation. On the other hand, the possibility remains that an occasional $+/mi$ heterozygote, particularly on a black or agouti background, may not be recognizable by its eye colour. That this can actually happen has recently been proved; in an F_2 mating to be described below, an agouti mouse was obtained which, from its extensive tail spotting (see next section), was expected to be of the constitution $+/mi$, but had deeply pigmented eyes like $+/+$; on testing, this mouse has proved to be $+/mi$, as suggested by its spotting. Similar cases not recognized by subsequent breeding tests may have occurred, but it is believed that their number must be small. On a chocolate and particularly on a cinnamon background a similar kind of overlap is most unlikely to occur.

SPOTTING IN MICROPHALMIA HETEROZYGOTES

The gene mi in heterozygous condition may behave as a spotting gene. This was discovered accidentally in an outcross of one of the three original animals from Hertwig's colony (a cinnamon $+/mi$ male, Mi 3) to two females from the grey-lethal stock. Had anything of the kind been anticipated, adequate records would have been kept from the start; as it happened, the three animals of the P generation were no longer in existence when the effect was discovered, and it remains unknown whether any of them showed minor spotting. Moreover, the grey-lethal stock has since turned out to segregate for one or more minor spotting genes and would thus not have been chosen for a critical analysis of such factors

The grey-lethal stock is homozygous for the normal alleles of all the known colour genes except grey-lethal. It has been outcrossed twice to the pure line Strong CBA , but is not highly inbred. No attention had been paid in the past to minor spotting genes in the stock. Of 159 normals in the stock recently classified, 114 showed no tail spotting whatsoever; thirty-four had unpigmented tail tips less than one-tenth of the total tail length, nine had tail tips between one-tenth and one-fifth of the tail in length, and two had tail tips about one-quarter long. No white spotting on belly or head has ever been observed in the stock. The grey-lethals in the stock show rather more tail spotting than their normal sibs, but there is no indication that the grey-lethal gene acts as a spotting gene in heterozygous condition. The data at hand seem to indicate that the strain is not homogeneous for the minor spotting genes which it carries.

In the F_1 generation produced by the outcross mentioned above, most of the $+/mi$ heterozygotes showed unpigmented tail tips of varying lengths, while the $+/+$ homozygous normals, so far as they were still available, showed no signs of tail spotting. The F_2 generation produced by inbreeding of F_1 animals consists of two kinds of mating; the greater part are $+/mi \times +/mi$, but there are also some $+/mi \times +/+$ matings which were

Table 3. *The distribution of spotting in the F_1 and F_2 generations derived from the outcross*

? indicates that the presence of spotting is uncertain; - =spotting absent; +=tail spotting; ++=spotting on tail and on head or belly.

Mating	+/+			+/mi				mi/mi	Total
	?	-	+	?	-	+	++		
F_1 Mi 3/Xa	4	6	.	.	10
Mi 3/Xb	8	6	.	.	1	8	.	.	23
Total	12	6	.	.	1	14	.	.	33
F_2 Mi 10/11	1	21	1	4	1	41	0	21	99
Mi 14/13	.	14	.	.	2	13	11	14	54
(A) Mi 18/27	.	14	.	.	2	20	8	21	65
Mi 30/33	.	14	3	.	.	14	16	18	65
Mi 36/37	.	16	1	.	.	26	10	31	84
Mi 38/39	.	26	5	.	1	14	18	21	85
Total	1	105	10	4	6	128	72	126	452
F_3 Mi 12/13	8	3	1	8	1	2	.	.	23
Mi 14/15	12	3	.	8	4	2	.	.	29
(B) Mi 18/26	4	13	.	3	1	4	1	.	26
Mi 19/27	7	1	.	1	3	4	1	.	17
Mi 30/31	.	4	.	.	1	4	1	.	10
Mi 32/33	3	4	.	5	.	.	2	.	14
Total	34	28	1	25	10	16	5	.	119

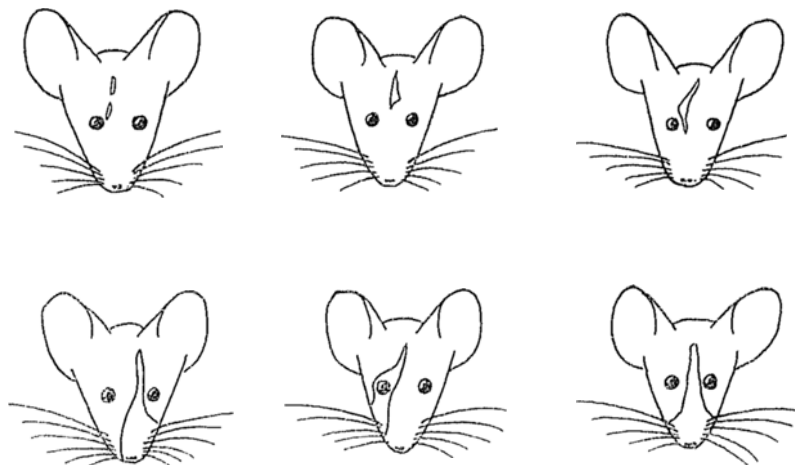


Fig. 6. Head spotting in $+/mi$ heterozygotes.

set up in the early days of the experiment when $+/mi$ could only be recognized by the offspring produced and not by their phenotype. Table 3 shows that in both types of F_2 generations the large majority of $+/+$ show no tail spotting, while the large majority of $+/mi$ are spotted. But, while in the F_1 generation tail spotting only occurred, there are numerous F_2 $+/mi$ animals which, in addition to tail spotting, show spotting on head, or belly, or both. The head spotting (see Fig. 6) is always near the midline of the head and varies from a few white hairs on the forehead through small head dots, and thin head

streaks to extensive wedges which include a large part of the whisker area. The belly spots are also generally strictly around the midline of the belly or chest and vary from small thin streaks in the umbilical or sternal region through streaks which are sometimes very long as compared with their width, to large flecks covering up to a quarter of the belly and chest area. Almost without exception (see, however, p. 10 below) spotting starts on the tail tip; head and belly spots do not occur unless tail spotting is also present. Spotting elsewhere on the body has never been observed.

The *mi* gene is not a spotting gene in its own right. This follows from the fact that in Hertwig's original strain tail spotting or spotting elsewhere does not seem to occur at all. Two of the three animals originally imported (Mi1♂ and Mi2♀) were of the constitution $a/a, b/b, +/d, +/mi$. Among the offspring of this pair obtained without outcrossing, thirty-four $+/+$ and ninety-six $+/mi$ mice have been examined for the presence of spotting. None of them showed as much as a trace of it. Hence it must be concluded that the spotting observed in the outcross is due to the interaction of $+/mi$ with a gene or genes introduced from the grey-lethal stock; unless the original $+/mi$ male Mi3 whose phenotype as to spotting is unknown carried such genes himself.

For reasons mentioned above, a complete and critical analysis of the case is impracticable with the data at hand. Certain broad conclusions can, however, be arrived at with reasonable assurance. Let us assume that the original male Mi3 carried no 'spotting modifiers' like the rest of the original strain bred without an outcross. Then the spotting observed in $F_1 +/mi$ must have entered the cross with the grey-lethal stock females. As it showed in heterozygous condition, the gene or genes concerned must be either dominant or semi-dominant. The occurrence of a more extreme phenotype (involving head and belly spotting) in F_2 suggests that tail spotting in F_1 represents the heterozygous condition, while the more extreme types of spotting include homozygotes for the gene(s) involved.

Some deductions concerning the number of spotting 'modifiers' involved can be made from the data of Table 3. Concentrating on the larger F_2 generation (A of the table), if the appearance of spotting in $+/mi$ depended on a single semi-dominant gene, one-quarter of the $+/mi$ in F_2 should be devoid of spotting (assuming that both grey-lethal stock females carried the same spotting genes, which may not be correct). Actually, only six out of a total of 206 $+/mi$ mice classified for spotting lacked tail spotting. Hence it is quite certain that more than a single gene is involved. If two pairs of genes were involved, $\frac{1}{16}$ or 12.9 mice should be devoid of spotting, whereas only six were actually found. Even assuming that these six unspotted mice were indeed all genetically self and did not include any normal overlaps as to spotting, the fit with expectation would be distinctly poor; actually, as there was one self mouse out of fifteen $+/mi$ in F_1 , which was presumably a normal overlap, the same probably applies to some of the $F_2 +/mi$ selfs. From this it may be concluded that the lowest plausible number of genes involved would be three; this leads to an expectation of one sixty-fourth or 3.2 self $+/mi$ mice in F_2 which fits the observed segregation reasonably well. It is, however, quite possible that even more pairs of genes were involved in this cross.

On the other hand, the F_2 (B) generation in Table 3 shows a much higher proportion of self $+/mi$ animals. The reason for this is by no means clear, particularly as six F_1 animals contributed to both F_2 generations.

Some of these deductions have been tested by breeding F_3 and F_4 generations from the F_2 just discussed (see Tables 4 and 5).

It was assumed above that the animals which have head and/or belly spotting in addition to tail spotting represent homozygotes for the 'spotting modifiers' involved. That this assumption is substantially correct can be seen from four F_3 and F_4 families in which both parents were 'extensively' spotted. Sixty-five out of sixty-nine $+/mi$ offspring produced were again 'extensively' spotted, though in some of them the head or belly spotting was only slight; the four remaining animals are presumably normal overlaps as to head or belly spotting.

The nature of some of the 'spotting modifiers' can also be deduced from Tables 4 and 5. Mating Mi 38/39 in Table 3 produced several $+/+$ animals with fairly extensive tail spotting; when these were first observed, it was thought that perhaps these might be

Table 4. F_3 generation

Origin (Table 3)	Mating	Spotting type	$+/+$		$+/mi$			mi/mi	Total
			-	+	-	+	++		
Mi 10/11	Mi 46/47	$+ + \times +$	11	5	.	22	8	10	56
Mi 10/11	Mi 48/49	? \times ?	2	.	.	7	3	3	15
Mi 12/13	Mi 50/51	$- \times +$	16	1	7	9	2	14	49
Mi 38/39	Mi 56/57	$+^* \times + +$	3	10	.	5	9	.	27
Mi 38/39	Mi 62/63	$+^* \times + +$	9	18	.	7	26	.	60
	Total		12	28	.	12	35	.	87
Mi 30/33	Mi 66/67	$+ + \times + +$	12	1†	.	.	33	18	64

* These two animals were homozygous normals with tail spotting; all other animals used for breeding in this table were $+/mi$.

† This $+/+$ had a small belly spot, but no tail spotting.

Table 5. F_4 generation

Origin (Table 4)	Mating	Spotting type	$+/+$		$+/mi$			mi/mi	Total
			-	+	-	+	++		
Mi 46/47	Mi 70/71	$+ + \times +$	5	9	.	13	16	18	61
Mi 56/57	Mi 77/78	$+ + \times + +$.	6	.	2	15	3	26
Mi 66/67	Mi 82/79	$+ + \times + +$	1	6	.	1	7	13	28
Mi 62/63	Mi 80/81	$+ + \times + +$	7	.	.	1	10	6	24

$+/mi$ mice in which the reduction of eye pigment was too slight to be noticeable; this suspicion was supported by the fact that the mating in question was producing a significant excess of $+/+$ mice as compared with the expectation. To test this point, two of the spotted animals in question were mated to undoubted $+/mi$ sibs with 'extensive' spotting. The resulting matings Mi 56/57 and Mi 62/63 of Table 4 show that the animals under test were in fact $+/+$, in accordance with their eye colour, as no mi/mi young were produced. However, there is now an increase in spotting both in $+/+$ and in $+/mi$ young; amongst the former, twenty-eight out of forty mice showed tail spotting, though to a much slighter extent than their $+/mi$ sibs. The latter now show a much higher proportion of 'extensively' spotted animals than did the corresponding F_2 family. It thus follows that some, at least, of the genes which produce spotting in the $+/mi$ mice have a similar, though much slighter, effect in $+/+$ animals. Hence they are spotting genes in their own right and cannot be classified as 'specific modifiers' in the strict sense.

On the other hand, it can be shown that this does not apply to all the genes involved. In matings Mi 66/67 (Table 4) and Mi 80/81 (Table 5) all but one of the forty-four $+/mi$ offspring are 'extensively' spotted; on the other hand, all but one of their twenty $+/+$ sibs are completely devoid of spotting. These animals obviously have the same genetic

background as the spotted $+/mi$ mice; but, as it does not result in spotting, we must conclude that the gene or genes involved do not produce spotting by themselves, but only in association with the gene for mi in heterozygous condition. Hence they are 'specific modifiers' in the strict sense.

It seems that there are at least two different specific modifiers which can bring about spotting in $+/mi$ heterozygotes. In all the cases so far mentioned in this paper, the head or belly spotting of $+/mi$ was associated with tail spotting; there was not a single $+/mi$ mouse which had belly or head spotting in the absence of tail spotting. A different situation was observed in mating Mi 54/55 (not included in the above tables) in which the male Mi 54 had a short unpigmented tail tip (class 2, see below) while the female Mi 55 had a tiny tail tip (class 1) associated with a white streak over the sternal region. The offspring produced was as follows:

$+/+$		$+/mi$			mi/mi	
-	+	-	+	++		Total
18	0	2	4	23	11	58

As all the $+/+$ are devoid of spotting, while nearly all the $+/mi$ are spotted, we are again dealing with specific modifiers. But in this case the 'extensively' spotted class includes five mice which have various degrees of head spotting, while the tail shows no spotting whatever. Clearly a gene with a different spotting *pattern* is involved in this family. The family Mi 54/55, it should be explained, is an F_3 derived from the outcross of Mi 1♂ to a female of the grey-lethal stock; Mi 1♂ is the second male imported from Hertwig's colony; the results of that outcross (which was made on a much smaller scale) are generally similar to the data given here in detail, with the exception of the family just mentioned.

To obtain at least semi-quantitative data about the degree of tail spotting, the length of the unpigmented tail tip (together with that of unpigmented bands which appear on some tails) has been recorded as a fraction of the whole tail. The following arbitrary classes are distinguished:

Class	Fraction of tail unpigmented
1	Less than $\frac{1}{10}$
2	$\frac{1}{10}$ - $\frac{1}{5}$
3	$\frac{1}{5}$ - $\frac{2}{5}$
4	$\frac{2}{5}$ - $\frac{3}{5}$
5	$\frac{3}{5}$ and over

Table 6 shows that generally the *degree* of tail spotting found in $+/+$ mice is much smaller than that occurring in $+/mi$ mice, and that this still applies when the general degree of spotting is increased, as in the last two matings. The remainder of the data, not included in Table 6, adds nothing of analytical interest.

Table 6. *Semi-quantitative data on tail spotting*

Genotype	...	$+/+$					$+/mi$				
Spotting class	...	5	4	3	2	1	5	4	3	2	1
F_1	1	4	4	1	2
F_2 A	.	.	1	.	3	6	8	69	66	37	14
F_2 B	1	.	2	5	1	2
Mi 46/47	.	.	.	1	2	2	1	13	14	1	.
Mi 50/51	1	1	6	2	2	.
Mi 56/57
Mi 62/63	.	.	3	5	6	14	17	22	6	2	.

Note. For some of the F_1 and F_2 animals, no quantitative data are available.

A more detailed and critical analysis of the spotting effects initiated by the *mi* gene would require very much larger experiments involving the use of homogeneous stocks. No such attempt has been made; it is indeed doubtful whether the results of such an investigation would repay the trouble.

DISCUSSION

The genes for microphthalmia and for grey-lethal form a remarkable set of 'mimic' genes. So far as the lack of bone absorption is concerned, grey-lethal is the more extreme of the two; as to the degree of pigment reduction and the presence of ocular involvement microphthalmia is more severe. Neither in one gene nor in the other is there an obvious physiological link between the reduced or lacking bone absorption and the reduced or lacking melanin formation. That such a link must nevertheless exist is strongly indicated by the fact that a reduction of pigmentation is associated with each of the two genes known to reduce bone absorption. Similar considerations obviously apply to other sets of 'mimic' genes whose pleiotropic syndromes have not yet been resolved into a 'pedigree of causes' (Grüneberg, 1948).

The lack of bone absorption is complete in the grey-lethal under normal physiological conditions; the normal stimuli for bone absorption are insufficient to set the process going. That even a grey-lethal can absorb bone under extreme stimulation has been shown by Barnicot (1945); bone absorption takes place as a result of massive parathyroid hormone injections which kill normal mice. In the microphthalmic mouse, bone absorption obviously takes place in response to some normal stimuli, but not in response to others. A comparative study of the grey-lethal and the microphthalmic skeleton should thus furnish some information concerning the normal stimuli of bone absorption and their relative efficiency. Investigations of this kind are now under way.

The absence of pigment in the *mi/mi* mouse might be interpreted either as a case of generalized pigment reduction (in analogy with albinism) or as a case of extreme spotting. Strong arguments can be adduced for either interpretation. The eye pigment reduction in the $+/mi$ heterozygotes is as clearly a dilution effect as the head dots, belly spots and tail tips of many of the heterozygotes constitute spotting. The *mi* gene is thus a combined dilution and spotting gene and resembles in this respect the viable allele of dominant spotting (W^v ; Little & Cloudman, 1937) and the varitint-waddler gene (Cloudman & Bunker, 1945) in the mouse, both of which show white spotting, but in addition reduce the intensity of the pigment in those areas of the fur where pigment is formed. In a sense, the yellow gene (A^y) also belongs to this category, as in addition to its effect on coat colour, this gene *reduces* the amount of white spotting in $W/+$; $+/s$ and $W/+$; s/s (variegated and 'black-eyed white') animals (Dunn, MacDowell & Lebedeff, 1937). In view of this growing list of genes which affect simultaneously the quality or quantity of pigment per hair ('hair colour') and the ability or inability of certain body areas to form pigment ('spotting'), it becomes increasingly clear that the sharp distinction made between 'colour' and 'spotting' genes by some earlier writers has torn open a gap which does not in fact exist.

The spotting situation described for $+/mi$ heterozygotes in this paper is similar in several respects to that in 'dominant spotting' ($W/+$) in the mouse (Dunn, 1937). Neither gene is a spotting gene in its own right. Whether and how much spotting will appear in $W/+$ heterozygotes is determined by a set of specific modifiers ($m(W)$ complex

of Dunn) which do not produce spotting by themselves, but do so in the presence of $W/+$. The existence of similar specific modifiers responsible for spotting in $+/mi$ heterozygotes has been made very probable in this paper; they were obviously absent from Hertwig's original stock, but present in the grey-lethal stock to which outcrosses were made. Whether the specific modifiers of $+/mi$ are identical with the $m(W)$ complex of Dunn is unknown. The fact that the spotting pattern produced by $m(W)$ in $W/+$ (a diffuse roan effect or variegation) is very different from the near-symmetrical spotting around the midline of the body in $+/mi$ mice does not necessarily prove that different genes are involved.

In addition to these specific modifiers, the spotting in $+/mi$ mice is partly conditioned by genes which produce a slight spotting effect by themselves, but whose effect is considerably increased in $+/mi$ heterozygotes. Such minor spotting genes are very common in mice and have been described on several occasions (for instance by Grüneberg, 1936*b*). With regard to such a minor spotting gene, *mi* could legitimately be described as a 'specific modifier', as it has no spotting effect of its own, but considerably enhances the spotting effect of the 'main' gene. A somersault such as this clearly illustrates the absurdity of the present nomenclature involving 'main' and 'modifying' genes. Any such distinction is purely arbitrary and in many cases positively misleading.

SUMMARY

The gene for microphthalmia in the mouse described by Hertwig shows a greatly reduced secondary bone absorption throughout its skeleton in the homozygous condition. It closely resembles in this respect, and in the reduction of pigmentation, the grey-lethal mouse in which the failure of bone absorption is complete. The two genes are, however, not allelomorphic to each other; it remains uncertain whether they are carried in different chromosomes.

In heterozygous condition, the gene for microphthalmia dilutes the choroidal pigmentation of the eye. The separability of the heterozygotes from $+/+$ normals is practically complete in the eight genotypes resulting from the combination of the genes *A v. a*, *B v. b*, and *D v. d*.

Depending on the genetic background in which it finds itself, the gene for microphthalmia in heterozygous condition may act as a spotting gene. Spotting results in the presence of certain 'specific modifiers' which do not produce spotting by themselves. In addition, the *mi* gene also enhances the effect of certain minor genes which produce tail spotting in their own right. The spotting starts on the tail tip and in more marked cases spreads to the belly and/or the forehead; it always keeps in close proximity to the midline of the body. In the absence of these genes, $+/mi$ shows no spotting whatever; the gene is thus not a spotting gene in its own right.

The bearing of the case on certain questions of pleiotropic gene action, on the distinction between 'colour' and 'spotting' genes, and between 'main' and 'modifying' genes is briefly discussed.

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