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A gene triplet in the mouse*

By A. G. Searle and Gillian M. Truslove
M.R.C. Radiobiology Unit, Harwell, Berks. and
M.R.C. Experimental Genetics Unit, University College London

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SUMMARY

Mice heterozygous for rump-white (*Rw*) have white hair in lumbo-sacral and caudal regions, although the tail-tip is sometimes pigmented. The homozygote is lethal *in utero*. No recombination has been found between *Rw* and the very closely linked spotting genes patch (*Ph*) and the viable allele of *W* (W^v). The compounds between these genes are all viable and fertile, although individual homozygotes are either lethal (*Ph*, *Rw*) or sterile and anaemic (W^v). It is concluded that they are non-allelic, but form a gene triplet. Close linkage between a cluster of dominant spotting genes and an angora gene in mouse and rabbit provide evidence for homology of part of linkage group II in the rabbit and part of linkage group XVII in the mouse.

1. INTRODUCTION

Although closely linked genes with similar phenotypic effects have frequently been described in *Drosophila* and micro-organisms, yet only a few examples have been found so far in mammals. One striking example in mice has been the close proximity of the spotting gene patch *Ph* to the well-known locus for dominant spotting and macrocytic anaemia comprising the *W* series of alleles (Grüneberg & Truslove, 1960). The present paper describes another dominant spotting mutation which behaves as if non-allelic with either *Ph* or the viable allele of *W* (W^v) but is clearly very closely linked to them, thus forming a gene triplet. This new mutation has been called rump-white, with symbol *Rw*.

2. GENETICS

(i) *Origin and phenotype*

The rump-white mutation arose in the course of a radiation experiment in which hybrid mice were given a dose of 214 rads fast neutron irradiation ($+\gamma$-contamination) over a period of twelve weeks and then mated to a specific locus stock (Batchelor, Phillips & Searle, 1966). Among the 24 dominant mutations recovered was one which caused the carrier female to lack hair pigmentation in the hinder parts of the body. Such rump-white mice (Plate 1) have white tails, apart from frequent

* In memory of Dr Margaret M. Dickie who died on 4 July 1969.

distal pigmentation, white hind-legs and a variable area of depigmentation in the sacral and lumbar regions. This tends to be rather more extensive ventrally than dorsally. Occasionally there are islands of pigmented hair surrounded by white. Digits of forefeet also tend to be white. In males, however, the scrotal skin remains dark even though scrotal hair and surrounding skin and hair are unpigmented, while in females the perianal skin is also pigmented. When the tail is partly pigmented the tip usually has black hairs as well as skin, but proximal to this there is an area of pigmented skin with unpigmented hairs. Pigmented areas of the coat show no signs of dilution.

Thus rump-white shows a marked phenotypic resemblance to patch *Ph* (Grüneberg & Truslove, 1960), except that the main area unpigmented in the latter mutant is in the thoraco-lumbar rather than the lumbo-sacral region. However, the feet, and much of the tail are white as in *Rw*/+, although the average amount of tail pigmentation is greater in patch. It is interesting to note that when the ventral white area includes part of the scrotal region in *Ph*/+ males the scrotum itself lacks pigment, unlike the situation in *Rw*.

(ii) *Segregation and linkage*

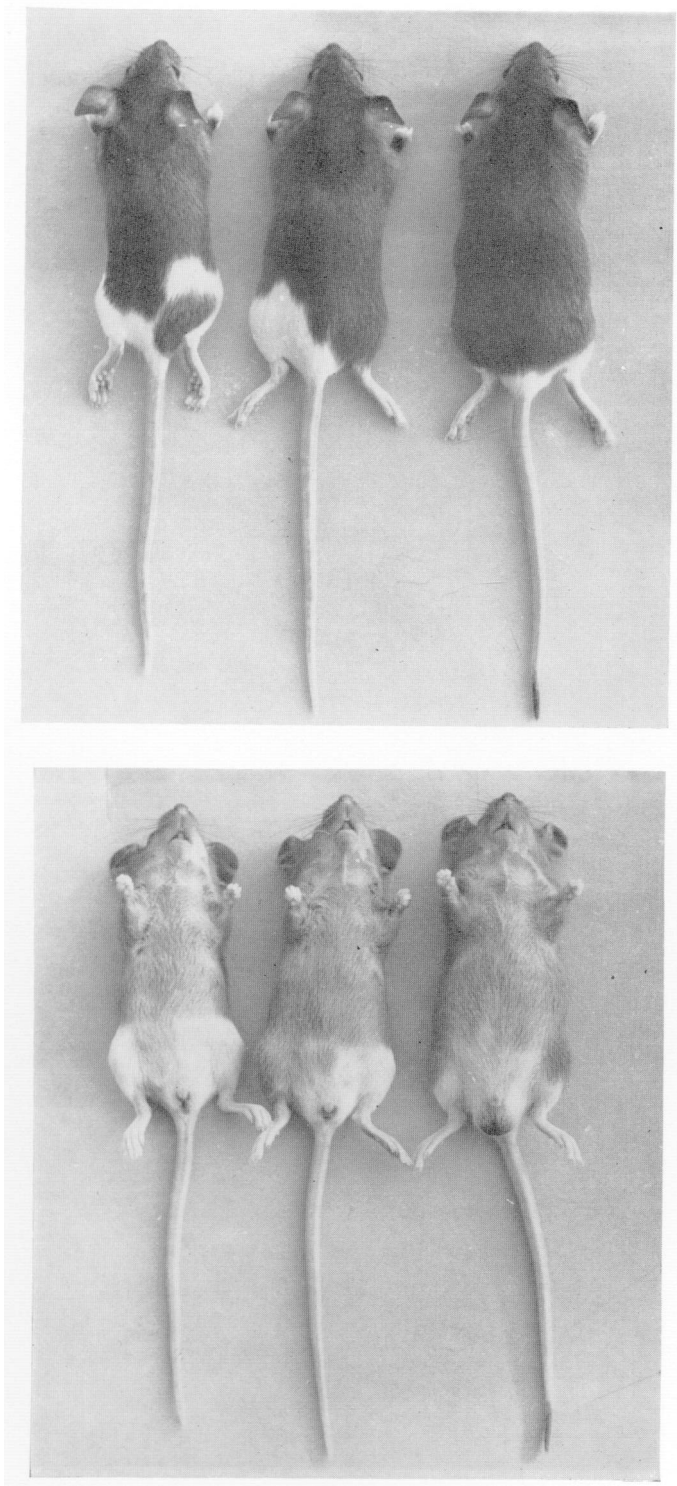
Rump-white behaves like a fully penetrant dominant mutation which is lethal in the homozygote, since it gives a good 1:1 ratio on outcrossing but a 2:1 rather than a 3:1 ratio on intercrossing (Table 1). The divergence from a 3:1 ratio is significant ($\chi^2_1 = 7{\cdot}3$, $P < 0{\cdot}01$). Dissections of pregnant females from *Rw*/+ intercrosses revealed an excess of dead embryos in mid-pregnancy, at about the frequency expected if they comprised the *Rw*/*Rw* class.

Table 1. *Segregation of rump-white*

Type of mating	*Rw*/+	+/+	Total	% *Rw*/+
Rw/+ × +/+	470	478	948	49.6
Rw/+ × *Rw*/+	185	90	275	67·3

Rw is very closely linked to *Ph* and W^v in linkage group XVII. Repulsion backcross matings of the double heterozygotes have so far produced no recombinants at all in the progeny (Table 2). Reasons why the genes are not considered allelic are given later. The classified F_1 progeny from the linkage test preparatory matings of *Rw* and W^v heterozygotes showed the following numbers of the four phenotypes: 155 *Rw*/+, 166 W^v/+, 175 +/+ and 136 *Rw*+/+W^v. With similar crosses of *Rw* and *Ph* numbers were: 175 *Rw*/+, 182 *Ph*/+, 197 +/+ and 146 *Rw*+/+*Ph*. Thus frequencies of the double heterozygotes were less than expected in each F_1, which suggests that they may have been at a slight disadvantage. In the *Rw*/+ × *Ph*/+ cross the divergence from a 1:1:1:1 ratio was significant ($\chi^2_3 = 7{\cdot}86$, $P < 0{\cdot}05$).

The upper fiducial limits ($P = 0{\cdot}05$) for map distances are 0·4 cM for *Rw* and W^v, and 1·4 cM for *Rw* and *Ph*. *Ph* and W^v can be estimated as 0·08 cM apart,



Dorsal and ventral views of rump-white (*Rw*/+) mice with variable amounts of depigmentation. Note pigmented scrotum in male, which also has a pigmented tail-tip.

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since Grüneberg & Truslove (1960) found 1 recombinant in 1302 offspring from $Ph+/+W^v$ backcrosses.

The phenotypes of the three double heterozygotes between *Rw*, *Ph* and W^v are quite distinct (Text-fig. 1). In $Rw+/+W^v$ mice pigmented hairs are scattered among unpigmented ones in thoracic and lumbar regions as well as on the head. There is always a large mid-dorsal head-spot and very little ventral pigmentation. The pigmentation is much more restricted in the other two genotypes. In $Rw+/+Ph$ mice there is a sharp demarcation line between pigmented and white regions in the shoulder area and behind the throat; the pigmented hair looks of normal intensity. In $Ph+/+W^v$ mice (described by Grüneberg & Truslove, 1960) pigmentation is

Table 2. *Results of repulsion backcross matings*

I. With $Rw+/+W^v$

Sex of heterozygous parent	Phenotypes				Total
	$Rw+$	$+W^v$	$++$	$Rw\ W^v$	
♂	384	408	0	0	792
♀	121	130	0	0	251
Total	505	538	0	0	1043

II. With $Rw+/+Ph$

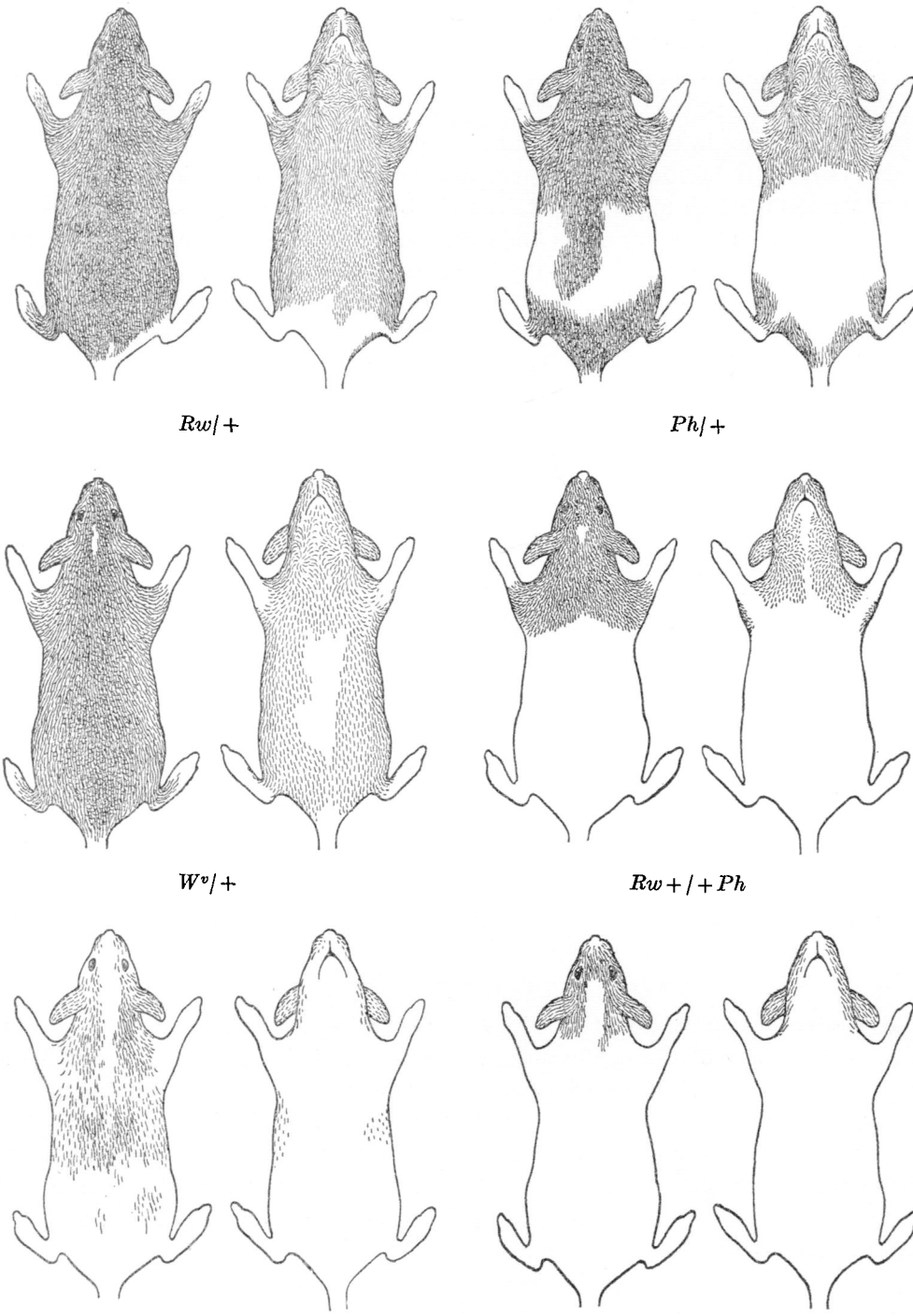
Sex of heterozygous parent	Phenotypes				Total
	$Rw+$	$+Ph$	$++$	$Rw\ Ph$	
♂	98	105	0	0	203
♀	30	24	0	0	54
Total	128	129	0	0	257

dilute and more or less entirely restricted to the head, which has a large mid-dorsal white area rather like that of $Rw+/+W^v$. Ears are pigmented in all three genotypes.

(iii) *Interactions with other genes*

Rw showed marked interaction with a number of other spotting genes. Generally the posterior part of the coat was much more affected than the anterior. Thus *Rw*/+ ; *s*/*s* mice had plenty of pigmentation remaining on head and shoulders, but little elsewhere. This contrasted with the phenotype of *Ph*/+, *s*/*s* mice which was either completely white or very nearly so (Grüneberg & Truslove, 1960). With lethal spotting *ls*, *Rw* showed a greater reduction of pigmentation than with *s*, so that only one or a few discrete patches of colour were left, mainly dorsal and anterior to the sacro-caudal region.

Text-fig. 2 shows the typical appearance of compounds between *Rw* and belted *bt*, the Harwell repeat of steel-Dickie Sl^{d-H} and Splotch *Sp*. With *bt*, as with *s*, the area without pigmentation seems less than Grüneberg & Truslove (1960) found for *Ph*. A comparison of *Ph*/+ ; Mi^{wh}/+, with *Rw*/+ ; Mi^{wh}/+ phenotypes reveals a similar situation, since in the former pigment is almost entirely



Text-fig. 1. A comparison of the appearance of *Rw*/+, *Ph*/+ and W^v/+ mice and of the three compounds between them.

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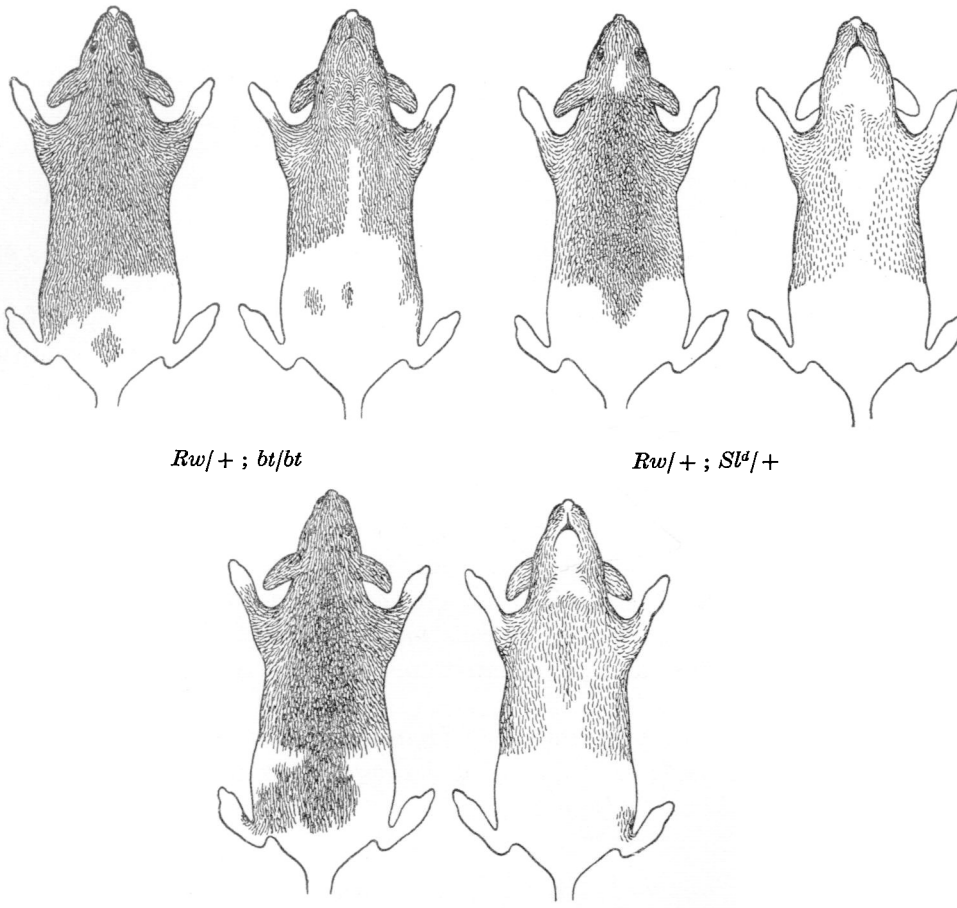

Rw/+ ; *bt*/*bt*

Rw/+ ; *Sl*^{d}/+

Rw/+ ; *Sp*/+

Text-fig. 2. The appearance of compounds between *Rw* and three other spotting genes: *bt*, *Sl*^{d} and *Sp*.

confined to head, shoulders and fore-legs (Grüneberg & Truslove, 1960), while in the latter the thoracic region is usually pigmented too.

(iv) *Distribution of pigment cells*

Thin slices of skin were taken from the dorsal lumbo-sacral region of 1 to 3 day-old rump-whites and normal sibs which had been killed and fixed in 10 % formalin. They were cleared and mounted in Canada balsam in a well-slide and examined microscopically to see the distribution of pigmented melanocytes. At 1 day the larger hair bulbs already contained pigment cells full of melanin granules in both phenotypes, except in the rump region of the presumptive *Rw*/+ in which bulbs were unpigmented but otherwise seemed normal. Pigmented dendritic melanocytes could also be seen scattered throughout the epidermis in this region. They were clearly visible even in areas of the *Rw* mouse which were devoid of pigmented

hair-bulbs, in much the same density as in the normal litter-mate. By 3 days these dendritic cells had formed a network over the whole area in both phenotypes.

Dorsal skin slices from 1- and 2-day patch mice were also examined and compared with normal and rump-white litter-mates. Very few pigmented epidermal melanocytes were seen in *Ph*/+, either in regions of pigmented or of white hair. In the pigmented rump region there were decidedly fewer than in normal litter-mates or in white rump areas of *Rw*/+ mice.

There was no very sharp demarcation line between areas of pigmented and non-pigmented hair-bulbs in the *Rw*/+ mice examined, for unpigmented hair-bulbs were sometimes surrounded by pigmented ones, and vice versa. Skin-slices from *Rw*/+; *ln*/*ln* and +/+; *ln*/*ln* litter-mates were examined in order to compare numbers of pigmented melanocytes (clumped by the action of *ln*) in hair-bulbs of the two genotypes. No differences were found, neither was there any sign of a decrease in melanocyte numbers in hair-bulbs as the white area was approached.

(v) *Blood picture*

Table 3 shows the red-blood pictures in *Rw* heterozygotes and compounds with closely linked genes. Haemoglobin was estimated by means of a Unicam spectrophotometer; the volume of packed cells was determined with a Hawksley microhaematocrit centrifuge and cell counts were made with a Coulter counter. Clearly

Table 3. *The red-blood picture of 13–14 day-old animals:* (*I*) Rw, Ph *and compound compared with normal;* (*II*) Rw, W^v *and compound compared with normal. The averages are based on 11 mice per group*

I	+/+; +/+	*Rw*/+; +/+	+/+; +/*Ph*	*Rw*/+; +/*Ph*
Hb (g/100 ml)	8·3	8·5	8·1	8·5
R.B.C. per mm³	5067000	5120000	5041000	4982000
Haematocrit (%)	29·0	29·6	30·2	29·4
Mean corpuscular volume in μ^3	57.7	57·8	60·8	59·3
Mean Hb concentration (%)	28·6	29·3	27·2	28·9
Mean Hb content in $\gamma\gamma$	16·5	16·5	16·4	17·2

II	+/+; +/+	*Rw*/+; +/+	+/+; +/W^v	*Rw*/+; +/W^v
Hb (g/100 ml.)	8·6	8·8	8·5	8·0
R.B.C. per mm³	5297000	5319000	4606000	4215000
Haematocrit (%)	30·8	31·2	30·9	29·5
Mean corpuscular volume in μ^3	58·7	59·1	67·6	70·1
Mean Hb concentration (%)	28·0	28·2	27·6	27·3
Mean Hb content in $\gamma\gamma$	16·4	16·6	18·6	19·1

Rw heterozygotes have a normal blood picture, as do *Ph* heterozygotes (see also Grüneberg & Truslove, 1960) and the *Rw*+/+*Ph* compound. The macrocytosis and low R.B.C. count in W^v heterozygotes agrees with previous findings by Grüneberg (1942) and others. The double heterozygote of *Rw* and W^v is slightly more affected than W^v/+ alone with respect to both the R.B.C. count ($t = 2{\cdot}44$, $0{\cdot}05 > P > 0{\cdot}02$) and the mean corpuscular volume ($t = 0{\cdot}86$, $P \sim 0{\cdot}4$) and

closely resembles the $Ph+/+W^v$ phenotype in this respect (Grüneberg & Truslove, 1960).

(vi) *Skeletal variation*

Grüneberg & Truslove found that the interfrontal bone in $Ph/+$ mice was often exceptionally large. Table 4 shows that in *Rw* the interfrontal is significantly more often absent than in normal litter-mates (χ^2_1 with Yates's correction = 7·4,

Table 4. *Size of interfrontal in rump-white and normal litter-mates. Arbitrary classes, with (+) representing those in which the bone is only visible from inside the skull*

	—	(+)	+	++	+++	++++	Total	% absent
+/+	7	10	6	16	9	2	50	14·0
Rw/+	18	8	4	8	6	0	44	40·1

Table 5. *Skull measurements in mm of rump-white and normal litter-mates. A = skull width at fronto-maxillary sutures; B = minimum skull width in frontal region; C = skull length from posterior margin of basi-sphenoid to nasal spine*

Measurement	*Rw*/+	+/+	*t*	d.f.	*P*
A	4·78	4·84	1·32	11	0·2
B	3·75	3·76	0·64	11	0·5
C	15·17	15·45	1·80	11	0·1

Table 6. *Numbers of lumbar vertebrae (left/right) in rump-white and normal litter-mates*

	6/6	6/5	5/6	5/5	Total	% 6/6
Rw/+	23	5	0	16	44	52
+/+	25	6	0	19	50	50

Differences clearly not significant.

$P = 0{\cdot}007$), but its size when present is little affected. The effect in patch mice was found to be associated with increased width of the skull in the anterior frontal region. The same skull measurements as those done on $Ph/+$ mice were made on *Rw*/+ and normal litter-mates, but no significant differences were found (Table 5). Neither were any significant differences found in the position of the lumbo-sacral border in the two genotypes (Table 6).

3. DISCUSSION

So far, *Rw* has shown no recombination with either *Ph* or W^v, yet there is good evidence to exclude allelism or pseudo-allelism with either of these genes, for the following reasons. When combined with W^v, all known *W* alleles give a severely anaemic black-eyed white phenotype which, when viable, is sterile. Yet $Rw+/+W^v$ mice merely show an increased amount of white with an R.B.C. count only slightly lower than in W^v heterozygotes and with full fertility. Also, *Ph* and *Rw*

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homozygotes both die *in utero*, yet *Rw*+/+*Ph* mice are fully viable. Like *Rw*+/+*W*^{v}, they show a striking interaction effect. However, such effects between quite independent spotting genes are well known in mice and occur between *Rw* and undoubtedly independent genes, as we have seen.

Another possibility which ought to be considered in view of the homozygous lethal action of both *Ph* and *Rw* is that they represent small deficiencies, probably not overlapping with each other (since the compound is fully viable) nor with the *W* locus itself (since they do not behave like *W*-alleles). *Rw* was probably a radiation-induced mutation and it is well known that ionizing radiation can induce deficiencies as well as true base-pair mutations (see Sobels, 1969). However, known deficiencies in the *d-se* region of linkage group II of the mouse, which have been extensively studied by L. B. and W. L. Russell (1960) do not seem to have any phenotypic effect in the heterozygote apart from possible size reduction in some. Deficiencies with phenotypic effects in the heterozygote are known in *Drosophila* (e.g. Notch) but are rare.

Grüneberg & Truslove (1960) showed that it was highly improbable that two spotting genes would come to lie in close proximity on the same mouse chromosome just by chance; clearly it is even less probable for three spotting genes to do so. Thus it would seem logical at present to regard *Ph*, *Rw* and the *W* series as a gene triplet which has arisen by repeated duplication of the original chromosome segment. It seems probable that the events concerned took place in the remote past, since the functions of the components of the triplet show marked differences.

From this point of view, a striking similarity between mouse and rabbit genomes should be pointed out. In the rabbit, the genes for Dutch (*du*) and English (*En*) spotting are only about 0·2 units apart, while the recessive gene for angora (*l*) is about 13 units away (see Robinson, 1958). In the mouse, the spotting-genes *Ph* and *Rw* are very close to the *W* locus, while the recessive gene for angora (*go*) is about 7 units away (Dickie, 1966). The Dutch and patch phenotypes resemble each other in tending to have a belt of white fur around the body, while the *En*/+ phenotype resembles that of some *W* series heterozygotes. *En*/*En* rabbits are less severely affected than *W* series homozygotes both in the extent of depigmentation and with respect to viability: nevertheless, there is good evidence that the former do survive less well than heterozygotes or homozygous wild type (see Robinson, 1958). Thus it seems very probable that part of linkage group II in the rabbit and linkage group XVII in the mouse are homologous.

Several other examples of the close association of genes with similar effects are known in the mouse. These include naked (*Na*) and shaven (*Sha*) which are 0·8 cM apart (Flanagan & Isaacson, 1967) and which both cause hairlessness in the homozygote, also fused (*Fu*), kinky (*Ki*) and the brachyury (*T*) series of alleles, which are close together on linkage group IX and show similar skeletal abnormalities (see Green, 1966). In addition, three esterase loci (*Es*-1, *Es*-2 and *Es*-5) are situated close together on linkage group XVIII (Petras & Biddle, 1966; Popp, 1967).

The presence of many extra-follicular dendritic melanocytes in the white rump

region of *Rw*/+ mice shows that the lack of hair pigmentation in this region is not due to any complete failure of melanoblasts to migrate into it. The presence of macroscopically visible skin pigmentation in scrotum and tail reinforces this view. Therefore the most probable explanation for the lack of hair pigmentation is that pigment cells reach the rump epidermal region too late to enter the developing hair-bulbs. Alternatively, the error may lie in the developing follicle rather than in the migrating melanoblast.

In *Ph*, however, regions of unpigmented hair also lack visible skin pigmentation in the adult and epidermal melanocytes in the young. Thus their white areas may be the result of a somewhat different mechanism from that in *Rw*, but further detailed studies are needed to clarify the situation.

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