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A presumed deletion covering the *W* and *Ph* loci of the mouse

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SUMMARY

A new allele at the *W*-locus (W^{19H}), found in a mutagenesis experiment in which females were irradiated, involves a presumed deletion. The deletion covers the *Ph* locus (which forms part of a gene complex with the *W*, *Ph* and *Rw* loci), and the locus of a recessive lethal 2 cM distal to *W*. It does not extend distally to the *bl* locus; nor does it involve the *Rw* locus, W^{19H}/Rw compounds being viable and fertile. Thus, the length of the deletion is 2–7 cM. The non-involvement of *Rw* shows that, in the gene triplet *Rw*, *W*, *Ph*, *Rw* must lie proximal to *W* and *Ph*, whose relative position remains unknown. Heterozygotes for W^{19H} are not anaemic, show only minimal white spotting and no pigment dilution; they thus resemble heterozygotes for the original *W* mutant allele and differ from *W*/*Ph* trans heterozygotes, which have extensive white spotting. In addition W^{19H} heterozygotes may be small and runted, many are believed to die prenatally, and some in the nest. Their radiosensitivity is increased. Homozygotes die at the pre-implantation stage.

1. INTRODUCTION

A wide variety of mutant alleles at the *W*-locus have been reported, (E. S. Russell, 1979; Geissler, McFarland & Russell 1981) with effects of varying degrees of severity on pigmentation of the coat, haematopoiesis, viability and fertility. The allele reported here is believed to be the first involving a presumed deletion of the *W* locus. Furthermore the deletion appears to cover the *Ph* locus, which forms part of a gene complex involving the *W*, *Ph* and *Rw* loci (Searle & Truslove, 1970).

The full effects of the *W*-locus include white spotting in the coat, sometimes accompanied by dilution of the remaining pigment, macrocytic anaemia which may be lethal in homozygotes, undue radio-sensitivity, and in homozygotes lack of mast cells and sterility due to near absence of germ-cells. Mutant alleles at the *Ph* locus also result in white spotting, and have effects on haematopoiesis and radio-sensitivity (Loutit & Cattanach, 1983); homozygotes for mutant alleles die in utero sometimes having a 'split-face' malformation. The *W* and *Ph* loci lie very close together with only a few rare crossovers ever having been reported (Grüneberg & Truslove, 1960; Geissler *et al.* 1981). The *Rw* locus also is very close to *W* and *Ph* (Searle & Truslove, 1970) and affects pigmentation, heterozygotes for the mutant allele having a white rump. The homozygote is also lethal prenatally.

The allele to be reported here, designated W^{19H}, appears to involve deletion of the *W* and *Ph* loci, but not the *Rw* locus, together with a > 2 cM segment distal to *W* and *Ph*.

2. ORIGIN

The original mutant animal was found among the offspring in an experiment in which female mice were given a dose of 4·0 Gy X-rays and mated so as to sample germ-cells irradiated as mature oocytes (Lyon, Phillips & Fisher, 1979). The mutant mouse was a male with minor white spotting. When mated with a normal hybrid female (C3H/HeH × 101/H) F_1 he produced several offspring with similar minor spotting. The mode of inheritance appeared to be autosomal dominant, and the phenotype of the heterozygotes resembled that of heterozygotes for some *W* alleles. In order to test for allelism with *W*, a putative $W^{19H}/+$ male was crossed with a female homozygous for the viable *W*-allele W^{sh} (Lyon & Glenister, 1982). This mating produced, in addition to typical $W^{sh}/+$ heterozygotes, a class of black-eyed white animals, which were of rather low viability in that 11 of 37 died before weaning age. These black-eyed whites were presumed to be W^{sh}/W^{19H} compound heterozygotes, and this was confirmed by crossing the surviving animals to wild type. Of 97 classified offspring (Table 1) 23 were typical $W^{19H}/+$, 72 typical $W^{sh}/+$ heterozygotes, and two appeared wild-type. The interpretation was that W^{19H} and W^{sh} were allelic, and that the two apparent wild-type animals were $W^{19H}/+$ animals showing incomplete penetrance.

3. GENETICS

Crosses of $W^{19H}/+$ with wild-type yielded the expected two classes of offspring, $W^{19H}/+$ and $+/+$, but there was a marked deficiency of $W^{19H}/+$ (Table 1). When $W^{19H}/+$ heterozygotes were crossed together only $W^{19H}/+$ and $+/+$ offspring were found and it was concluded that W^{19H}/W^{19H} homozygotes died prenatally. Again, the observed proportion of $W^{19H}/+$ was much less than expected. Some W^{19H} offspring were small and runted, and some died before weaning age. In addition, the percentage of young dying in the nest before classification of white spotting was, at 12·6%, higher than normal in our stocks. Part of the deficiency of W^{19H} offspring was therefore probably due to death before classification. However, the number of recorded deaths was inadequate to account for all of the deficiency. Some $W^{19H}/+$ animals had very little white spotting, and it was therefore suspected that a further part of the deficiency might be incomplete penetrance. This was supported by the two apparent wild-type young found among the offspring of W^{sh}/W^{19H} animals mentioned above. However, these same offspring of W^{sh}/W^{19H} suggested that failure of penetrance was quite low, and again inadequate to account for all of the deficiency of $W^{19H}/+$.

To investigate further the shortage of $W^{19H}/+$ offspring, the litter-sizes at birth of litters sired by W^{19H} males were compared with those of litters sired by males carrying other *W*-alleles, showing normal segregation. The two *W*-alleles used were W^{28H} and W^{29H}, both of which arose in a mutagenesisis experiment using ethylnitrosourea; W^{28H} is lethal when homozygous and W^{29H} viable. Females

mated to all the males were C3H/HeH × 101/H F_1, and to avoid any bias due to change in litter-size with litter-order the comparison was limited to the first eight litters of females which had eight or more litters. The mean litter-size of seven females mated to $W^{28H}/+$ or $W^{29H}/+$ males was $7{\cdot}84 \pm 0{\cdot}38$, and that of a similar 7 females mated to $W^{19H}/+$ males was $5{\cdot}59 \pm 0{\cdot}32$. The difference is statistically highly significant ($t = 4{\cdot}49$, $P = 0{\cdot}000017$). As the females in the two groups were

Table 1. *Genetic tests with* W^{19H}

Cross	Offspring			
	$W^{sh}W^{19H}$	$W^{sh}+$	$W^{19H}+$	$++$
$W^{sh}/W^{sh} \times W^{19H}/+$	37	71	–	–
$W^{sh}/W^{19H} \times +/+$	–	72	23	2
$W^{sh}/+ \times W^{19H}/+$	21	34	19	34
$W^{19H}/+ \times +/+$			31	75
$+/+ \times W^{19H}/+$			130	430
$W^{19H}/+ \times W^{19H}/+$			28	40
	$W^{19H}W^{x}$	$W^{x}+$	$W^{19H}+$	$++$
$W^{19H}/+ \times W^{e}/+$	0	15	4	17
$W^{19H}/+ \times W^{v}/+$	9*	17	5	20
	$W^{v}W^{19H}$	$W^{v}+$	$W^{19H}Ph$	$+Ph$
$W^{19H}+/++ \times W^{v}+/+Ph$	8*	31	–	29
	$W^{19H}Rw$	$+Rw$	$W^{19H}+$	$++$
$W^{19H}+/++ \times ++/+Rw$	12	35	25	41
$W^{19H}+/++ \times$ T264$+/+Rw$	2	6	5†	10
$W^{19H}+/+Rw \times ++/++$	–	27	10	–
	$W^{sh}W^{19H}$	$W^{sh}+$	$W^{19H}+$	$++$
$W^{19H}+/++ \times W^{sh}+/+l$	9	15	–	16
$W^{19H}+/++ \times W^{sh}l/++$	–	47	13	34
$W^{19H}++/++ \times +blgo/+blgo$			7	22

Data from the first two crosses were previously reported by Lyon and Glenister (1982).
* Only one survived to weaning age
† Two proved to carry T264.

comparable the lower litter-size with $W^{19H}/+$ males suggests that there was excess prenatal or perinatal loss in this group. The ratio of the litter-sizes is 5·59/7·84 or 0·71. If it is assumed that the whole of the excess loss in the W^{19H} matings is due to death of $W^{19H}/+$ offspring, then the proportions of surviving young are $0{\cdot}5+:0{\cdot}21\,W^{19H}$. In other words, only 42% of $W^{19H}/+$ offspring survive to birth. The observed proportion of $W^{19H}/+$ surviving at classification from matings of this type was 130/430 or 30·2%. Thus, the further postnatal loss (42–30 = 12%) is small compared with the perinatal loss.

Crosses were next made to other *W* alleles, and to *Ph* and *Rw* (Table 1). Presumed W^{v}/W^{19H} compounds were detected as black-eyed white anaemic young in the nest, but only 2 of 17 survived to weaning age. One survived to adulthood and was anaemic and sterile. No apparent W^{e}/W^{19H} offspring were detected, and it was

presumed that this genotype died prenatally. Unexpectedly, there were also no recognizable $W^{19H}+/+Ph$ offspring, indicating prenatal death of this genotype also. Animals of the type $W^{19H}+/+Rw$ were, however, found, and were viable; three males tested proved fertile (Table 1). (The number of offspring of this type was below expectation on the basis of equal proportions of $W^{19H}+/++$ and $W^{19H}+/+Rw$, and the reason for this is unknown).

The apparent prenatal death of $W^{19H}+/+Ph$ suggested that W^{19H} involved a deletion covering the loci of both *W* and *Ph*. In order to test this, crosses were made to stocks carrying a recessive lethal, *l*, located 1–2 cM distal to *W* (Lyon & Glenister, 1982). Two stocks of this lethal were available, carrying W^{sh} either on the same, or on the homologous chromosome. When crosses of $W^{19H}+ \times W^{sh}l/++$ were made no black-eyed white $W^{19H}+/W^{sh}l$ young were born but some $W^{19H}+/++$ were obtained, and conversely crosses of $W^{19H}/+ \times W^{sh}+/+l$ yielded some $W^{19H}+/W^{sh}+$ but no $W^{19H}+/+l$ offspring (Table 1). From this it was concluded that the presumed deletion extended distally from *W* to include the locus of *l*.

To estimate the extent of the deletion W^{19H} was tested against mutants at more distally sited loci. The recessive mutants blebbed, *bl*, and angora, *go*, are situated at 7 and 8 cM distal to the *W* locus. If the W^{19H} deletion extended to these loci one would expect that the W^{19H} offspring of a cross of $W^{19H}/+ \times blgo/blgo$ would be phenotypically blebbed or blebbed angora. In fact, such a cross yielded only typical $W^{19H}/+$ and wild-type offspring (Table 1). It was concluded that the deletion did not extend to the locus of *bl*. The reciprocal translocation T(5; 13)264Ca (T264) is lethal when homozygous and has a breakpoint 4 cM distal to *W* (Searle, 1981) and thus about 2 cM distal to *l* and between *l* and *bl*. Therefore, $W^{19H}/+$ animals were crossed to $T264+/+Rw$ to test whether $T264+/+W^{19H}$ offspring were viable. If this class were not viable then this cross would yield phenotypically $W^{19H}+/+Rw$ animals, but only very rare $W^{19H}/+$ animals (resulting from crossing-over between T264 and *Rw*). There were in fact 5 $W^{19H}/+$ young, compared with only 2 $W^{19H}+/+Rw$, and both of two $W^{19H}/+$ animals genetically tested were proved to carry T264. This test thus provides no positive evidence that the W^{19H} deletion extends to the T264 breakpoint. However, it is possible that the lethality of T264 is due to a lesion in chromosome 13, rather than chromosome 5, and thus the possibility that the W^{19H} deletion extends to the T264 breakpoint is not excluded.

On the proximal side of *W*, the *Pgm-1* locus, which lies 3 cM from *W*, was studied. For this test 11 $W^{19H}/+$ animals were tested for electrophoretic polymorphism of *Pgm-1*. Of these animals, 6 were phenotypically PGM-1AB and 5 PGM-1A. The presence of the PGM-1AB animals shows that both homologues of chromosome 5 carried a *Pgm-1* allele, and hence that the locus of *Pgm-1* was not deleted in W^{19H}. The PGM-1A animals indicate that the allele present on the W^{19H} chromosome was $Pgm\text{-}1^a$; this is consistent with the origin of W^{19H} by mutation in a (C3H/HeH × 101/H) hybrid genetically $Pgm\text{-}1^b/Pgm\text{-}1^a$.

4. DESCRIPTION

Heterozygous $W^{19H}/+$ animals typically show minor degrees of white spotting. Usually there is a white spot or patch on the belly, the feet are white, and the tail is at least partially white. Sometimes there is in addition a white blaze on the forehead, and also in some cases a white half-belt. Some $W^{19H}/+$ animals have had white on the feet and tail only, with no belly or head spotting. The pigmented parts of the coat have full wild-type colour, with none of the pigment dilution seen in W^{v} heterozygotes.

Table 2. *Dissections of pregnant females carrying* W^{19H}

Parents			CL	Imp	Live embryo	Dead		Imp	Live
Female	Male	No.	(per ♀)	(per ♀)	(per ♀)	embryo	Moles	CL	Imp
+/+	$W^{19H}/+$	9	127 (14·1)	98 (10·9)	87 (9·7)	2	9	0·772	0·888
$W^{19H}/+$	+/+	9	90 (10·0)	79 (8·8)	60 (6·7)	4	15	0·878	0·759
$W^{19H}/+$	$W^{19H}/+$	9	85 (9·4)	46 (5·1)	40 (4·4)	–	6	0·541	0·870

CL, corpora lutea; Imp, implantations.

Some $W^{19H}/+$, as mentioned above, are small and runted, and some die before weaning age, of unknown causes. Other animals are of normal size and fertility.

The RBC count of $W^{19H}/+$ is within the normal range, and the cells are not macrocytic. In $W^{19H}+/+Rw$ there was a marginal reduction in RBC count (Loutit, in preparation). Despite the normal RBC count, radiosensitivity of $W^{19H}/+$ is increased, the median lethal dose being 5–6 Gy, compared with a normal value of ~ 8 Gy (Loutit, Corp & Adams, 1982).

In order to determine the time of death of the prenatally lethal W^{19H}/W^{19H} homozygotes, dissections were made of pregnant females at $13\frac{1}{2}$ to $17\frac{1}{2}$ days gestation, and the numbers of corpora lutea, implants, and live and dead embryos were counted (Table 2). The $W^{19H}/+$ females had slightly fewer corpora lutea per female than normal females, whether mated to wild-type or to the $W^{19H}/+$ males. When mated to wild-type males the proportion of corpora lutea represented by implants was as high as that in normal females, and the proportion of implants surviving as live embryos was again not significantly lower than in the reciprocal cross. However, when $W^{19H}/+$ females were crossed to $W^{19H}/+$ males the proportion of corpora lutea represented by implants was markedly reduced, as too was the number of implants per female. Conversely, the proportion of implants surviving as live embryos was not reduced. The excess preimplantation loss in $W^{19H}/+ \times W^{19H}/+$ crosses was $1-(0{\cdot}541/0{\cdot}878)$ or 38·4 %, and was thus entirely adequate to account for the expected 25 % loss of lethal W^{19H}/W^{19H} homozygotes. It was therefore concluded that the W^{19H} homozygotes died at the preimplantation stage.

The dissections provided little information concerning the time of death of the missing $W^{19H}/+$ heterozygotes discussed above. In the reciprocal crosses of

$W^{19H}/+ \times +/+$ there was no obvious excessive loss of embryos, either pre-implantation or postimplantation up to the stage of the dissections. On the other hand the number of live embryos per female in the matings of $+/+$ ♀ $\times$ $W^{19H}/+$ ♂ was $9{\cdot}7 \pm 0{\cdot}37$, and thus considerably higher than the mean litter-size at birth from crosses of this type of $5{\cdot}59 \pm 0{\cdot}32$. This discrepancy suggests that the death of $W^{19H}/+$ was occurring either late in gestation or at the time of birth.

5. CYTOGENETICS

Attempts were made to visualise the deletion by studies of Giemsa banded chromosomes from bone marrow. The translocation break in T264Ca lies in band E2 of chromosome 5 (Searle, 1981). Therefore, any visible deletion should have been apparent in or just proximal to this band. In fact no difference could be detected between the two chromosomes 5 of $W^{19H}/+$ heterozygotes.

6. DISCUSSION

The evidence seems to indicate clearly that W^{19H} involves a deletion. The minimum possible length of chromosome deleted is that between *W* and *l* and thus about 2 cM, and the maximum is from *W* to just proximal to *bl*, or 7 cM. On this basis the failure to observe the deletion cytogenetically is not surprising. The average length of a mouse chromosome is about 75 cM and chromosome 5, being one of the larger chromosomes, would be expected to have a greater length than this. Thus, a deletion of 2 cM would be less than 3% of the length of the chromosome, and this would be undetectable.

The deletion was induced by X-ray treatment of mature oocytes. L. B. Russell (1971; 1983) has shown that after such treatment a high proportion of the recessive specific locus mutations recovered are due to deletions. It is thus not surprising that a dominant visible mutation such as W^{19H} should also be due to deletion.

The phenotype of heterozygotes for W^{19H} might be considered surprising, in that there was very little white spotting. In trans heterozygotes of the type $W+/+Ph$, a high proportion of the body fur is white, leaving mainly the head and shoulders fully pigmented (Grüneberg & Truslove, 1960; Lyon & Glenister, 1982). Yet in $W^{19H}/+$, with the *W* and *Ph* loci deleted from one homologue of chromosome 5, only minimal white spotting occurs. This appears to be a confirmation of the views of Kacser & Burns (1981) who argued on theoretical grounds that mutations, such as deletions, leading to absence of a protein would be less likely to lead to a dominant effect, than those leading to presence of an abnormal protein. A similar example is provided by the *t*-haplotype t^{h20}. This is lethal in combination with the dominant tail-mutant knobbly, Fu^{kb}, and is thought to include a deletion covering the locus of Fu^{kb}. However, t^{h20} heterozygotes do not have the kinked tail characteristic of Fu^{kb} heterozygotes (Lyon & Bechtol, 1977).

The phenotype of $W^{19H}/+$ heterozygotes may also give some clue as to the possible type of lesion present in the numerous other *W* mutant alleles. The effect in the heterozygotes of the different alleles may vary from extensive to very little white spotting, and may or may not include dilution of the remaining coat

pigment, with or without some effect on haematopoiesis (Geissler *et al.* 1981). The appearance of $W^{19H}/+$ would suggest that the classic, original *W* allele, with little white spotting, no pigment dilution, and no anaemia in the heterozygote is the type most likely to involve absence or null function of the protein coded by the *W* locus, with other alleles having some type of abnormal protein.

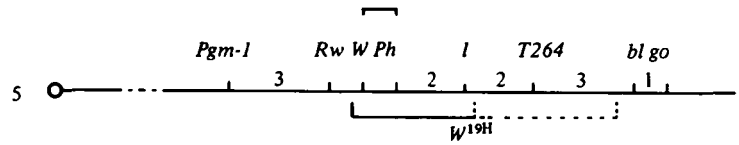

Figure 1. Diagrammatic representation of the region of chromosome 5 deleted in W^{19H}. The centromere is to the left. The solid line represents the known extent of W^{19H} and the dotted line the possible extent, between the loci of *l* and *bl*.

The preimplantation death of W^{19H} homozygotes is consistent with the expected effects of a deletion. Homozygotes for mutant alleles at known loci involved in the deletion, *W*, *Ph* and *l*, all die after implantation, at various stages and of presumably different causes. Thus the preimplantation death of W^{19H} must be due to some unknown locus in the deleted region, as also must be the late foetal or neonatal death of some $W^{19H}/+$ heterozygotes.

The deletion provides useful information concerning the order of the loci, *W*, *Ph* and *Rw*. Up to now these loci have been known to be very closely linked, but crossovers have been too few and too rare to establish the gene order. The W^{19H} deletion has been shown to extend distally 2 cM from *W*, to include the locus of *l*. However, it apparently does not cover *Rw*, $Rw+/+W^{19H}$ double heterozygotes being viable and fertile. This therefore implies that *Rw* must lie proximal to *W* and *Ph* (Fig. 1). However, the order of *W* and *Ph* relative to *Rw* cannot be determined.

It is hoped that the deletion may further prove useful as an aid to identifying the *W* gene, by DNA methods.

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