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SHORT PAPER

A recessive allele of the mouse agouti locus showing lethality with yellow, A^y

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SUMMARY

The radiation-induced agouti allele a^1 is recessive to the alleles a and a^{4H} (which resembles a^e). It is lethal when homozygous and also in combination with the dominant yellow allele A^y. The ethylnitrosourea induced allele a^{16H} is also lethal when homozygous, and when heterozygous with a shows a phenotype like that of a^x, with black back and lighter belly. Like a^x it is not lethal with A^y, and it is also not lethal with a^1. a^1 is believed to be the first recessive allele which is lethal with A^y, and may be useful in elucidating the complexity of the agouti locus.

1. INTRODUCTION

The lethal yellow allele, A^y, of the agouti locus of the mouse was one of the first lethal genes discovered in any species, the absence of homozygotes having been first reported by Cuénot in 1905 (reviewed by Silvers, 1979).

Since that date many mutations at the agouti locus have been noted and many new alleles reported, but very few reoccurrences of A^y, combining a yellow phenotype in the heterozygote with lethality in the homozygote, seem to have occurred. At least two further alleles giving a yellow coat colour in the heterozygote have been found, A^{vy} and A^{sy} (Silvers, 1979; Green, 1981), but both are viable when homozygous, the homozygotes having a yellow coat. A lethal allele, a^x, has also been reported (Russell, McDaniel & Woodiel, 1963; Papaioannou & Mardon, 1983) but this has a very different phenotype from A^y, and moreover the $A^y a^x$ heterozygote is viable.

We report here an allele, designated a^1 (and previously briefly reported by Phillips, 1976), which like a^x has a very different phenotype from A^y in the heterozygote, and is lethal not only when homozygous, but also in combination with A^y. It is not lethal in combination with a further allele similar in phenotype to a^x, and designated a^{16H}.

RESULTS

(*i*) *Origin*

The original mutant animal carrying a^1 was a male offspring, found in a mutagenesis experiment in which a C3H/HeH × 101/H F_1 (3H1) female mouse was treated with 600 cGy X-rays, and mated to a male homozygous for the seven recessives *a*, *b*, c^{ch}, *d*, *p*, *s* and *se*. The mutant appeared phenotypically *aa* (Lyon, Phillips & Fisher, 1979).

The original a^{16H} animal, a male, occurred among the offspring of a 3H1 male treated with 250 mg/kg ethylnitrosourea, mated to similar multiply recessive females, and bred

so that spermatogonial stem cells were sampled. The phenotype of the mutant differed from *aa* in that the belly and flanks were somewhat lighter, resembling agouti in colour. The dorsum of the animal was black, and the pinna hairs were yellow.

(ii) Genetic tests

Allelism of a^1 with the agouti locus was shown by a cross of the original, putative a^1a male to *aa*; all 15 offspring were non-agouti. In order to test for lethality, the original male was crossed to a 3H1 female (A^w+) and the offspring (all agouti, and genetically heterozygous either for a^1 or for a) were intercrossed. Three intercross pairs produced some phenotypically non-agouti offspring, but two pairs gave no non-agouti young, among totals of 47 and 76 respectively. The conclusion was that the genotype a^1a^1 was lethal, and that in those pairs which produced some non-agouti young one or both parents had carried the allele a, inherited from the mutation testing stock.

Lethality of the allele a^{16H} was tested in a similar way. The original mutant was crossed to 3H1 and offspring were intercrossed. One trio of two females and a male produced some non-agouti young, phenotypically resembling the original $a^{16H}a$ male, whereas one pair and a trio gave no non-agouti among totals of 47 and 43.

In further tests (Table 1), a^1 was crossed to the allele a^{4H}, which phenotypically resembles a^e, i.e. $a^{4H}a^{4H}$ homozygotes are coal black with black pinna hairs. Roughly half the offspring of crosses of a^1 heterozygotes to $a^{4H}a^{4H}$ (crosses 1 and 4, Table 1) also were coal black with black pinna hairs. These were concluded to be the a^1a^{4H} heterozygotes. Thus, a^1a^{4H} resembles a^ea^e.

The two new alleles a^{16H} and a^1 were then crossed together to test the viability of $a^{16H}a^1$ (crosses 4 and 5, Table 1). These matings yielded offspring with a new phenotype, mainly black with black pinna hairs, but with the agouti belly and flanks characteristic of a^{16H}. These animals were deduced to be genetically $a^{16H}a^1$. This was confirmed by a cross of putative $a^{16H}a^1 \times aa^1$ (cross 6), which yielded typical $a^{16H}a$ animals in addition to further $a^{16H}a^1$. In cross 7, putative $a^{16H}a^1$ animals were mated to a^ea^e. This again yielded a class of offspring with black pinna hairs and agouti belly and flanks; these were concluded to be $a^{16H}a^e$. Thus, the phenotypes of $a^{16H}a^1$ and $a^{16H}a^e$ were similar.

Both a^{16H} and a^1 were then tested for viability with A^y. When $a^{16H}a$ was crossed with A^ya (cross 8) there were 20 yellow and 22 non-yellow offspring. This good 1:1 ratio suggested that the A^ya^{16H} genotype was viable, and this was confirmed by genetic tests of the yellow offspring. Of 7 tested, 5 proved to be A^ya and 2 were A^ya^{16H}. Thus, A^ya^{16H} is viable and fertile. Conversely, among the offspring of $a^1a \times A^ya$ (cross 9), there were only 12 yellow to 19 non-yellow offspring, suggesting a deficiency of yellow. In more extensive data from $A^ya^{16H} \times a^ta^1$ (cross 10) the proportion of yellow offspring (31 out of 84) was significantly less than expected if A^ya^1 was viable ($\chi^2 = 6{\cdot}94$; $P < {\cdot}01$). Eleven yellow offspring from cross 10 were genetically tested, and all were A^ya^t, with none A^ya^1. It was therefore concluded that the genotype A^ya^1 was lethal.

Since both a^{16H} and a^1 are lethal the possibility arises that either or both involve a deletion. In order to test this point $a^{16H}a^1$ animals were crossed to *abp/abp* where *bp* (brachypody) is a marker very closely linked to the *a* locus, and showing only 0·4% recombination (Roderick & Davisson, 1981). Of 11 offspring of this cross, 5 $a^{16H}a$ and 6 a^1a, none showed any sign of *bp*, i.e. the locus of *bp* was not deleted in either a^{16H} or in a^1.

In crosses 3, 4 and 5 the segregation did not show good agreement with Mendelian expectation, but the reasons for this have not been investigated. In cross 3 there were fewer $a^{16H}a$ young than expected. This could have been due to misclassification as the agouti appearance of the belly of $a^{16H}a$ is in some cases little different from *aa*. However, other possibilities have not been excluded. In crosses 4 and 5 the discrepancy appeared to be mainly due to a shortage of $a^{16H}a^1$ offspring. Hence, it is possible that the viability

Table 1. *Results of genetic tests with the agouti allele* a^{16H} *and* a^{l}

Cross	Parents	Offspring			
1	$a^{l}a \times a^{4H}a^{4H}$	aa^{4H} 15	$a^{l}a^{4H}$ 12	—	χ^2 0·33
2	$+a^{l} \times a^{4H}a^{4H}$	$+a^{4H}$ 7	$a^{l}a^{4H}$ 11	—	0·89
3	$a^{16H}a \times a^{16H}a$	$a^{16H}a$ 45	aa 35	—	3·91
4	$a^{16H}a \times +a^{l}$	$+$ 70	aa^{l} 25	$a^{16H}a^{l}$ 14	11·04
5	$a^{16H}a^{e} \times a^{t}a^{l}$	a^{t} 28	aa^{l} 14	$a^{16H}a^{l}$ 6	4·0
6	$a^{16H}a^{l} \times aa^{l}$	$a^{16H}a$ 16	aa^{l} 14	$a^{16H}a^{l}$ 18	0·5
7	$a^{16H}a^{l} \times a^{e}a^{e}$	$a^{16H}a^{e}$ 5	$a^{l}a^{e}$ 6	—	0·9
8	$a^{16H}a \times A^{y}a$	A^{y} 20	aa 8	$a^{16H}a$ 14	1·8
9	$a^{l}a \times A^{y}a$	A^{y} 12	a 19	—	1·58
10	$A^{y}a^{16H} \times a^{t}a^{l}$	A^{y} 31	$a^{t}a^{16H}$ 30	$a^{16H}a^{l}$ 23	6·93
11	$a^{16H}a^{l} \times abp/abp$	$a^{16H}a$ 5	aa^{l} 6	—	0·9

of this genotype, carrying two complementing lethals, is not complete. However, in crosses 6 and 10 there appeared no clear shortage of this genotype, and thus the question of the full viability of $a^{16H}a^{l}$ must remain open.

3. DISCUSSION

The allele a^{l}, which shows the lethality of A^{y} when homozygous, but a very different phenotype when heterozygous, may throw further light on the nature of the agouti locus. Because of the numerous alleles, with a wide range of phenotypes, it has been suggested that the agouti locus is a complex one. Silvers (1979), in his review of this question, points out that the detailed suggestions for its various parts, with different genes coding for effects on back, belly and ears, do not seem entirely plausible. However, there is still the possibility of complexity in other respects.

A phenotype very similar to that of A^{y} can occur without lethality in A^{vy}. Similarly, a phenotype similar to a^{l} can occur without lethality in a^{e}. This suggests that the lethality may be due to a separate gene.

The A^{y} gene shows a complex syndrome of effects on characters other than coat colour. Heterozygous A^{y} animals become obese, with mild glycosuria, have increased susceptibility to neoplasia, and abnormal immunological responses (reviewed by Silvers, 1979). By use of the a^{l} allele it would be possible to test whether any of these effects are due to the lethal gene rather than the gene causing yellow coat colour. However, it

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already appears that most of the effects are due to yellowness per se, since similar effects are seen in animals with the viable yellow gene, A^{vy} (Silvers, 1979; Roberts, Wolff & Campbell, 1984). Furthermore, their severity is proportional to the amount of yellow hair in the coat of individual animals, which may vary in colour from yellow to almost wild-type agouti. Hence, it seems unlikely that the lethal gene is involved. Heterozygotes for a^1 do not appear obese, but the other effects have not been investigated.

As a^1 is a radiation-induced mutation it may well involve a deletion, both of the lethal locus and the coat colour locus. This is particularly so as it was induced in an oocyte, and Russell (1971) has shown that a high proportion of mutations induced by radiation in oocytes involve deletions covering more than one locus. Any such deletion would probably be small, as the nearby locus of *bp*, and the lethal locus in a^{16H} are apparently not involved.

The A^y allele is closely associated with an insertion of an ecotropic murine leukaemia virus, *Emv-15*, (Copeland, Jenkins & Kee, 1983). It is possible that this insertion has altered the gene involved in the lethality in such a way as to inactivate it, but not necessarily to delete it. The DNA lesion leading to the dominant yellow phenotype may also be an inactivation, of a gene affecting coat colour, but different from the one whose postulated deletion leads to the black phenotype of a^1. In this case A^y and a^1 would be functionally equivalent to two overlapping deletions. Alternatively, the dominant yellow phenotype might be due to a different lesion in the same coat colour gene as a^1. In this case, if the different phenotypes of the many distinct alleles of the agouti locus are due to changes in a single gene, then there must be a complex set of regulatory signals. There is already evidence for position effects acting on the agouti locus, in that the A^s and a^x alleles can cross over with other alleles of the locus, A^s with A^w and a^t (Phillips, 1966) and a^x with A^y (Russell *et al.* 1963). In fact, the phenotype produced by A^s now appears to be a position effect due to an inversion in (2)2H (Evans & Phillips, 1978). Susceptibility to position effects appears to imply the existence of cis-acting regulatory signals. Further details of the structure of the agouti locus will no doubt come from DNA studies and a^1 may be a useful tool in such work.

The allele a^{16H} is of interest for its resemblance to a^x. a^x was induced by radiation and a^{16H} by ethylnitrosourea. It will be of interest to compare the DNA lesions involved.

The work on a^1 was begun by Miss R. J. S. Phillips, who sadly died before its completion.

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