A CIS-TRANS POSITION EFFECT AT THE A LOCUS OF THE HOUSE MOUSE

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IN the mouse there are two types of hair pigment, eumelanin, the black or brown pigment, and phaeomelanin, the yellow pigment. A number of loci are known which affect the deposition of these two pigments, the most important of which is the agouti locus situated in linkage group V. There are 12 known alleles at this locus; A^Y yellow (see GRÜNEBERG 1952 and LOOSLI 1963); A^{vy} viable yellow (DICKIE 1962a); Intermediate yellow (A^{iy}) (DICKIE 1966); A^w light-bellied agouti; A agouti or wild type; A^i intermediate agouti (DICKIE 1962b); a^t black and tan; a^{td} tanoid (LOOSLI 1963); a^x (RUSSELL, McDANIEL and WOODIEL 1963); a non-agouti; a^e extreme non-agouti (HOLLANDER and GOWEN 1956) and a^m (RUSSELL 1964).

The agouti locus controls the deposition of the two types of pigment by affecting the environment of the melanocytes in the hair follicle (SILVERS 1961) but does not alter the pigment itself. It is a complicated locus acting in a sense independently on three sections of the body—the back, the belly and the pinna hairs together with certain hairs round the genital papilla and mammae—so that one allele may be dominant on the back and recessive on the belly or vice versa; basically, irrespective of this “pattern” effect, yellowness is dominant to black. For example the yellow banding of hairs on the back of A is dominant to the self-colour of a or a^t ; the yellow belly of a^t is dominant to the dark belly of A or a and the black hairs of the pinnae and round the genital papilla and mammae of a^e are recessive to the yellow hairs of these regions in all other alleles. A partial exception to this rule is found in the allele A^i where the heterozygote $A^i a$ is umbrous, that is, the yellow banding of the back of the homozygote $A^i A^i$ is only expressed on the flanks of an $A^i a$ animal, the non-agouti dominating in the central back region (DICKIE 1962b).

Presumed intragenic crossing over was found at the agouti locus by L. B. RUSSELL *et al.* (1963) with the lethal allele a^x . In the progeny of a balanced lethal yellow stock ($A^y/a^x \times A^y/a^x$) she found four normal wild-type (A) animals out of 1908 mice raised.

The mutant described in this paper, A^s , agouti suppressor, also shows crossing over with agouti; whether it is part of the agouti complex or independent of, but closely linked to, it will be considered in the DISCUSSION. It occurred amongst the offspring of a (C3H $\times$ 101) F_1 male which had received an acute dose of 600r

X rays (PHILLIPS 1961) and was originally thought to be a recessive allele of A (PHILLIPS 1959, 1960).

OBSERVATIONS AND RESULTS

Description of A^s : A^s when homozygous reduces the amount of yellow pigment normally produced by whatever agouti alleles are present. The yellow pinna hairs found with all alleles except a^e become black (or brown on a homozygous brown background etc.); with a^t/a^t the back and belly are black; with A^w/A^w the size of the normal yellow band and the percentage of banded hairs are reduced giving a very dark agouti effect shading to almost complete non-agouti on the top of the head (with A^w/a^t this "non-agouti" patch is rather larger, but the two genotypes are not positively distinguishable). The belly of the $A^s A^w/A^s A^w$ animal is also dark-agouti; the $A^s a^t/A^s a^t$ animal is undistinguishable from a^e/a^e .

Where A^s differs from other genes found in the mouse is that when it is heterozygous it seems to affect only the agouti allele situated on the same chromosome as itself (Table 1). For example $A^s a^t/+A$ is phenotypically normal wild-type and $A^s a^t/+A^w$ is normal light-bellied agouti, whereas $A^s A^w/+a$ is umbrous, dark-agouti bellied and $A^s A^w/+a^t$ umbrous and tan-bellied. In other respects A^s behaves in a normal Mendelian fashion and crossing over between it and the agouti locus can be shown.

Breeding results: A^s was originally thought to be an umbrous allele at the A -locus similar to A^i but has since been shown to cross over with A^w and a^t . The data are given in Tables 2, 3 and 4.

The crossover value is higher in males than in females, the opposite to that found for other genes in this region of linkage group V, notably between non-agouti and mahogany (LANE and GREEN 1960). This difference is not significant.

TABLE 1

Phenotypes of A^s in combination with other A alleles

Genotype	Corresponding phenotype		
	Back	Belly	Pinna hairs
$A^s A^w/A^s A^w$	Dark agouti*	Dark agouti*	Black
$A^s A^w/A^s a^t$	Dark agouti*	Dark agouti*	Black
$A^s a^t/A^s a^t$	Non-agouti	Non-agouti	Black
$A^s A^w/+A^w$	Normal agouti	Yellow	Yellow
$A^s a^t/+A^w$	Normal agouti	Yellow	Yellow
$A^s A^w/+A$	Normal agouti	Normal agouti	Yellow
$A^s a^t/+A$	Normal agouti	Normal agouti	Yellow
$A^s A^w/+a^t$	Umbrous†	Yellow	Yellow
$A^s A^w/+a$	Umbrous†	Dark agouti*	Yellow
$A^s A^w/+a^e$	Umbrous†	Dark agouti*	Black
$A^s a^t/+a^t$	Non-agouti	Yellow	Yellow
$A^s a^t/+a$	Non-agouti	Non-agouti	Yellow
$A^s a^t/+a^e$	Non-agouti	Non-agouti	Black

* Dark agouti animals have less yellow banding in their agouti areas.

† Umbrous animals—the centre back is non-agouti, the flanks dark agouti.

The standard errors are quoted with the linkage values in the various tables, but as the expected numbers in the recombination classes are less than 5 they are not very meaningful.

From the breeding results the following facts can be deduced: (1) A^s is not at the same point on the chromosome as A^w or a^t . (2) A^s combined with either of the agouti alleles tested (A^w and a^t) is recessive to its normal allele with A or A^w on the homologous chromosome. (3) A^s when combined with A^w and with its normal allele and a on the homologous chromosome interferes with the agouti effect such that non-agouti dominates in the centre-back region (giving an umbrous phenotype) but itself dominates on the flanks and belly giving dark agouti

TABLE 2

Data on the segregation of A^s , A^w and a^t ; A^s and a^t in repulsion

Genotype of parents	Genotypes and phenotypes of offspring					Total
	Back: Belly: Pinna hairs:	$A^sA^w/+a$	$A^sa^t/+a$	$+A^w/+a$	$+a^t/+a$	
		Umbrous	Non-agouti	Agouti	Non-agouti	
		Dark agouti	Non-agouti	Yellow	Yellow	
$A^sA^w/+a^t \text{ } \text{♀} \times +a/+a \text{ } \text{♂}$		132	..	..	138	270
$+a/+a \text{ } \text{♀} \times A^sA^w/+a^t \text{ } \text{♂}$		130	3	1	169	303
Genotype of parents	Back: Belly: Pinna hairs:	A^sA^w/A^sA^w	A^sa^t/A^sA^w	$+A^w/A^sA^w$	$+a^t/A^sA^w$	Total
		Dark agouti	Agouti	Agouti	Umbrous	
		Dark agouti	Yellow	Yellow	Yellow	
	Black	Yellow	Yellow	Yellow		
$A^sA^w/+a^t \text{ } \text{♀} \times A^sA^w/A^sA^w \text{ } \text{♂}$		195	2	176	373	
$A^sA^w/A^sA^w \text{ } \text{♀} \times A^sA^w/+a^t \text{ } \text{♂}$		60	..	63	123	

Combined linkage value A^s/a^t in repulsion 0.73% (standard error 0.3%).

TABLE 3

Data on the segregation of A^s , A^w and a^t ; A^s and a^t in coupling

Genotype of parents	Genotypes and phenotypes of offspring					Total
	Back: Belly: Pinna hairs:	A^sa^t/A^sa^t	$+a^t/A^sa^t$	A^sA^w/A^sa^t	$+A^w/A^sa^t$	
		Non-agouti Non-agouti Black	Non-agouti Yellow Yellow	Dark agouti Yellow Black	Agouti Yellow Yellow	
$A^sa^t/+A^w \text{ } \text{♀} \times A^sa^t/A^sa^t \text{ } \text{♂}$		220	1*	2	236	458
$A^sa^t/A^sa^t \text{ } \text{♀} \times A^sa^t/+A^w \text{ } \text{♂}$		278	1	2	284	565

* Recorded as dark bellied with some yellow in ears, and killed by mistake with classification unchecked. Not included in calculations.

Combined linkage value A^s/a^t in coupling 0.50% (standard error 0.2%).

TABLE 4

A^s/a^t linkage. Data from Tables 2 and 3 combined

Sex of segregating parent	Linkage value (percent)	Standard error
Female	0.44	(0.2)
Male	0.75	(0.3)
Combined	0.60	(0.2)

(with a^t instead of a on the homologous chromosome an umbrous tan-bellied animal is obtained) (Table 1).

Linkage tests were set up with brachypody (bp), but as the only available stock also contained pallid, leaden and pearl, the amount of information obtained was not sufficient to give any indication of the linkage value. One definite bp crossover in about 50 fully classifiable mice was obtained in which A^s and A^{10} stayed together indicating that bp does not lie between these two genes. Some data on the segregation of bp and a supplied by DR. M. F. LYON, are shown in Table 5.

DISCUSSION

The A locus of the mouse is complicated with its differing dominance relationships on the back, belly and ears, but, until recently evidence seemed to support the hypothesis of a single locus with simple alternative alleles (GRÜNEBERG 1952; LOOSLI 1963).

The first possible crossover between supposed A alleles was reported by WALLACE (1954), who found a non-agouti animal from a mating of the type $A a^t \times aa$. In this case mutation from a^t or A to a could not be excluded. However RUSSELL *et al.* (1963) found evidence that a^x crossed over with A^y with a frequency of about 0.5%; they also found a single agouti animal from an $A^y/a \times A^y/a$ mating and considered that A^y might be pseudoallelic to both a^x and a . Their data do not exclude the possibility that a^x or A^y might be at an independent locus very closely linked to A and partially masking its effect.

A^s like either A^y or a^x shows definite crossing over with alleles of the A locus. Is it in fact part of the A locus, or independent of it? Research in microorganisms has introduced entirely new concepts of the structure and function of the gene and A^s will be considered in the light of these findings. WELSHONS (1965) has related the concepts of microorganisms to an analysis of the Notch locus in *Drosophila* and found evidence that amorphic *Drosophila* mutants at least can be shown to behave in the same way as genes in microorganisms; he concludes that "the genes of macro- and microorganisms are similar".

Work on bacteria in the last decade has indicated that a gene should be considered as a length of DNA rather than a particular point on the chromosome,

TABLE 5
*Data on the segregation of a with bp **

Genotype of parents	Phenotypes of offspring								Total
	$Aa^t bp^+$	$Aa bp^+$	$a^t a bp^+$	$aa bp^+$	$Aa^t bp$	$Aa bp$	$a^t a bp$	$aa bp$	
$a^t +/a bp \times A +/a bp$	46	54	35	1	..	..	..	40	176
	$a^t bp^+$	$a^t bp$	$a bp^+$	$a bp$	..	..	..	..	
$a^t +/a bp \times a bp/a bp$	34	..	..	36	..	..	..	..	70
	$A bp^+$	$A bp$	$a bp^+$	$a bp$	..	..	..	..	
$A +/a bp \times a bp/a bp$	12	..	..	9	..	..	..	..	21

* Data kindly supplied by DR. M. F. LYON.
Combined linkage value a/bp 0.38% (Standard error 0.4%).

culminating in the findings of YANOFSKY *et al.* (1964), who showed that a linear sequence of nucleotides in a gene can specify the linear sequence of amino acids in a protein. The difference between alleles at a locus could therefore be due to defects at different points along this length rather than at the same point, thus allowing intragenic crossing over. The *cis-trans* test for distinguishing between nonallelic but epistatic genes and a complex locus with internal crossing over was originally devised for *Drosophila* by LEWIS (1951) who interpreted the *cis-trans* effect as an indication of duplication within the locus. This test is based on complementation between genes; normally with two independent loci, recessive genes complement each other so that the two configurations *cis* (*ab*/++) and *trans* (*a*+/+*b*) are of the same phenotype and are wild-type. However, sometimes with two closely linked recessives of similar function the *trans* configuration becomes mutant in phenotype and the *cis* remains wild type or nearly wild type. In other words, if the two components demonstrate this *cis-trans* position effect or lack of complementation, then they are not independent of each other and can be assumed to be part of the same cistron or functional unit (BENZER 1957). Other phenomena can complicate this effect, for instance dominance. In *Drosophila* carrying the dominant Stubble (*Sb*) and a recessive allele stubbloid (*sbd*), *Sb sbd*/++ gives a wild type and *Sb* +/+ *sbd* an enhanced mutant phenotype, more extreme than either *Sb*+ or *sbd sbd*. On the other hand with the dominant Star (*S*) and a recessive asteroid (*ast*) allele, although *S* +/+*ast* again gives an exaggerated phenotype, *S ast*/++ is not wild type but resembles *S*+ (CARLSON 1959). Furthermore, work with *Neurospora crassa* has shown that all alleles within a cistron, depending on their function, need not be noncomplementary (FINCHAM and CODDINGTON 1963).

Finally JACOB and MONOD (1961) demonstrated that the *cis-trans* position effect can also occur over a larger area than one cistron. It can occur between cistrons when the latter are controlled by a single operator in the first of the cistrons; the group of cistrons is then termed an operon. The *cis-trans* position effect is found between mutations of the operator itself and all other mutations within the operon; alleles from different cistrons within the operon, on the whole, complement each other. The operon has two characteristics which differentiate it; first the various alleles of the operator itself act in a coordinated manner on all the other mutants, and secondly JACOB and MONOD (1961) and WELSHONS (1965) found that lack of complementation may sometimes occur between alleles of different cistrons in the operon but only in one direction; that is the operon shows polarity. Mutations in the first cistron may stop the functioning of the whole operon, whereas mutations sited in the second cistron will only be able to block that and subsequent cistrons.

If the breeding data of A^s are considered, it is at once obvious that, although complicated by differing dominance relationships, the two configurations $A^s A^w/+a^t$ and $A^s a^t/+A^w$ differ in phenotype. On the back $A^s A^w/+a^t$ gives a part non-agouti, part dark-agouti coat, whereas $A^s a^t/+A^w$ gives a normal agouti coat. On the belly, both configurations give a yellow colour (owing to the dominance of $+a^t$ and $+A^w$), but considered separately $A^s a^t$ with $+a$ on the homologous

chromosome gives a non-agouti belly, whereas A^sA^w in the same situation gives a dark-agouti belly; in neither case can yellow belly manifest itself. These data also show that although the yellow bellies of Aa^t and A^wa are phenotypically equivalent (WALLACE 1965), the yellow bellies of a^ta^t and A^wA^w are not genotypically so. Underlying the belly effect is the banding or nonbanding of the allele affecting the back.

It seems therefore that A^s is part of the A complex forming presumably either a cistron or an operon and shows lack of complementation with A^w and a^t , that is with both the dorsal and the ventral effects. No definite evidence is available as to whether the rest of the A alleles, other than a^s or A^y , are alternatives at one point or are scattered along the locus.

Before considering the possibilities of whether the A complex is a cistron or an operon, it is helpful to survey what is known about the function of the A locus and of A^s itself.

The A locus has been studied by SILVERS (1961) who showed, by transplantation experiments, that if melanocytes of one A genotype migrate into follicles of a different A genotype, then they subsequently produce the pigment pattern of the follicle into which they have migrated. In other words, whether any particular melanocyte produces yellow or black pigment is dependent solely on the follicular environment in which it finds itself. CLEFFMANN (1963) found that mouse hairs, *in vitro*, can also be made to produce either yellow or black pigment depending on their environment and irrespective of their genotype. On first culturing, all genotypes produce black pigment, but yellow pigment production can be induced by additions of glutathione to the medium. But CLEFFMANN found a difference between the various hair types in their susceptibility to the environment. Unbanded hairs (A^y and a , a^t) have a steady threshold value after the first two days of pigment production. Non-agouti hairs have a low susceptibility and A^y a high susceptibility; that is A^y hairs need a lower concentration of glutathione in order to produce yellow pigment. Banded hairs (A^w and A), on the other hand, need a high concentration of glutathione to produce yellow pigment except during the time of band formation, when they have a period of high susceptibility, higher even than that of A^y (CLEFFMANN 1963, Figure 7).

SILVERS work has indicated that the action of the melanocytes, i.e. whether they produce black or yellow pigment, is unspecific and dependent on the follicular environment, whereas CLEFFMANN has shown that different A alleles vary in their reaction to an outside agent, glutathione.

The A alleles must therefore be concerned with the production of a follicle environment within which the unspecific melanocytes can function. It is known that *in situ* yellow pigment cells show the presence of sulfhydryls whereas black pigment cells do not (CLEFFMANN 1963), so that one may assume that those follicles which would have produced yellow pigment *in situ* do contain some sulfhydryls already and the differences in the amount of glutathione which had to be added to the cultural medium with the different A alleles, could be due to the need to bring these "naturally occurring sulfhydryls" up to a threshold below which the melanocytes are unable to manufacture yellow pigment. There is as

yet no evidence as to whether the A alleles are directly concerned with the production of sulphhydryls or whether the glutathione acts as an inducer or regulator of the A system, such that some other end product forms the necessary environment for yellow pigment production.

The action of A^s seems to be to switch to the production of black pigment all the melanocytes in completely yellow hairs such as on the belly and in the ears, but it is unable to overcome the banding and deposition of yellow pigment produced by A^w . If it is assumed that the yellow hairs of the belly and ears would react in a similar fashion to glutathione as do A^y hairs, then an effect like that caused by A^s could be attained by altering the sulphhydryl concentration of the follicle to a level lower than that required by A^y hairs but not below that required by banded hairs at the peak of band formation. This could also account for the lower percentage of banded hairs in $A^s A^w$ phenotypes and for the fact that, when present, the band tends to be narrower than normal. In other words, A^s could be having a regulatory effect on the other alleles causing either an alteration of the sulphhydryl level or the reaction of other alleles such as A^w and a^t to that level.

Table 6 shows the various effects of the A alleles except for A^{vy} and a^m ; these differ from the other A alleles in causing phenotypic mosaicism. The various patches in the coat resemble the effects of other alleles and the mosaicism might

TABLE 6

Table of phenotypes of various combinations of A alleles (each phenotype, where known, is indicated by three symbols referring to back, belly and pinna hairs respectively)

	A^y	A^{iy}	A^w	A^i	A	a^{td}	a^t	a^s	a	a^e
A^y	lethal	YYY	YYY		YYY		YYY	YYY	YYY	YYY
	A^{iy}	viable			Sooty yellow				*YYY- BBY	
	A^w	BYY			BYY	BYY	BYY	BYY	BYY	BYY
		A^i	BYY	BYY	BYY				UY Y	
			A	BBY	BYY	BYY	BBY	BBY	BBY	BBY
				a^{td}	U ^D YY				U ^D YY	
					a^t	NYY	NYY	NYY	NYY	NYY
						a^s	lethal	NPY		
							a	NNY	NNY	NNY
								a^e	NNN	NNN

KEY:

- B = Banded (Agouti)
 U = Umbrous
 U^D = Umbrous combined with dark-agouti
 Y = Yellow
 N = Non-yellow, unbanded hairs (i.e. black on black background).
 P = as for N but paler.
 * Varies in phenotype from A^{vy} -like to wild type.

be due to variegated position effects such as are found in *Drosophila*. Such evidence as is available for a^m cannot as yet rule out small rearrangements but has excluded somatic mutation and major rearrangements (RUSSELL 1964).

How can the phenotypes of the remaining A alleles be explained within the framework of the action of A^s as discussed above?

(1) The locus can be considered as divided into regions each of which is concerned with a separate part of the body.

WALLACE (1965) interprets the A locus as a complex of several closely linked "mini-loci", where the agouti patterns of different parts of the body, (back, belly and ears) are separately controlled but varyingly epistatic to each other. That the A alleles can be masked is known from data on other mutants affecting the black/yellow pigmentation and situated in other linkage groups such as Sombre (BATEMAN 1961); dark (FALCONER 1956, 1957); mahoganoid (LANE 1960); and non-agouti curly (PHILLIPS 1963) or in the same linkage group but not closely linked to A (mahogany-LANE and GREEN 1960).

On the other hand there are various difficulties to this explanation: (a) As shown earlier in this discussion, the yellow belly of $a^t a^t$ animals is not equivalent to that of $A^w A^w$ animals so that WALLACE's interpretation would need to be modified to a locus for banding and a locus for yellow belly rather than one for dorsal and one for ventral banding. (b) Amongst known alleles there is an absence of such combinations as black ears and belly with yellow back or black ears with yellow belly. The relationship of back, belly and ears, as information stands at present, is such that if the ears are dark then so is the belly and if the belly is dark then so is the back. (c) This interpretation would require several of the known mutations to be multiple events. A^{iy} , which mutated in the C3H/HeJ ($A A$) stock (DICKIE 1966) would involve two events; a^{dt} and A^i , which mutated in the C57BL/6J (aa) strain (five years apart) (LOOSLI 1963; DICKIE 1962b) also would both involve two events; finally a^e which mutated in an A stock would probably involve three events as HOLLANDER and GOWEN (1956) pointed out. (d) A^s shows lack of complementation with effects in all three regions, showing that they are not in fact controlled independently.

These difficulties can be at least partly overcome by postulating that the locus consists of an operon of two or more cistrons which affect these different regions of the body and which show the polarity typical of operons. In this case A^s which seems to act coordinately on the other A alleles tested, could be assumed to be an allele of the operator or to be in the first cistron, and to effect transcription through the rest of the complex, a^e could also be sited here, whilst a^{td} would be an allele in a subsequent cistron and only affect the transcription of a "banding" region. Two events would still be involved in the a^{td} mutation unless the alteration causing yellow belly also affected the transcription of the "banding" region. This explanation is in line with the polarity phenomenon of operons mentioned earlier, and with the polarity of the A locus such that when the ears are dark, so is the belly and when the belly is dark then so is the back. The picture of the A complex would then be

// operator / ears + belly region / banding/ . . .
 A^s a^e a^{td}

and A^i could be filled to this framework somewhere within the banded region such that only the centre back was affected. A^{iy} could be considered as a mutant in the first or second cistron which increased the transcription of the belly and banded regions.

Further data are needed on the relative positions of the various alleles within the locus before one could consider this as more than a formal possibility.

(2) Still considering the locus to be an operon of various cistrons it is possible to devise and to fit the A alleles into a scheme based on (i) structural genes (affecting the ears, belly and whole body) which produce substances necessary for yellow pigment production in melanocytes, and on (ii) regulatory-type genes which control the structural genes. These second types of genes could be situated at various points along the locus or operon and allow transcription of any subsequent structural genes (to give yellow hairs), cause transcription to be intermittent (to give banded hairs) or block transcription completely (to give black hairs): or the scheme could be completely reversed with the A alleles producing substances inhibiting yellow pigment production and their action being suppressed by the presence of sulfhydryls. As there is at present no experimental evidence for or against such an idea, it is only put forward as yet another possible method, in line with findings in microorganisms, for explaining the complexities of the A locus.

(3) Finally the locus could be considered as consisting of one cistron, the structural alleles of which act on the whole body but produce differentials between one region and another.

Those alleles known to show crossing over with the rest, e.g. A^s , a^e and/or A^y , could be considered as mutations of the operator controlling the cistron; the other alleles would be structural mutants affecting the amount of some substances, such as sulfhydryls, on which the production of yellow pigment within the follicle depends. Alleles such as a^e which could be envisaged as producing none of these substances are easy to explain, but more difficulty is encountered with the intermediate alleles a^{td} , A^i and A^{iy} .

The umbrous, dark-agouti effect on the dorsum of a^{td} animals mimics A^s . In the normal A^w animal, the band width and percentage of banded hairs increase on the flanks compared with the centre back; therefore the effect of a^{td} could be attained by a mutant of an A^w type which produced the substances concerned in yellow pigmentation over a shorter period; the separate determination of belly colour would be as found in A^w and a^t .

Conversely, the production of yellow pigment inducing substances over a longer, variable period could account for the A^{iy} phenotype. A^i , on the other hand, must be having its effect over the same period as A^w as A^iA^i is equivalent to A^w ; it is possible that A^i is an A^w allele with a slightly deficient product which is incapable of complete gene dosage compensation so that the heterozygotes A^ia is an intermediate as found in many enzyme systems in man (HARRIS 1963).

The position of a^e and A^y relative to the other alleles is uncertain. Both could

be explained as operator-type mutants; A^y as a mutant with an opposite effect to A^s and a^x as similar to A^s but more extreme, so that an $a^x A/+a$ animal allowed no yellow banding on the flanks and only lightened the belly. Alternatively, both could be explained as structural mutants increasing or decreasing the length of time available for yellow pigment production.

The mapping of a^x and A^y , which RUSSELL *et al.* (1963) determined as $kr-a^x-0.5\%-A^y-un$, makes it likely that a^x rather than A^y is crossing over with the other A alleles because

(a) RUNNER (1959) found that the map of this region was $kr-bp-0.3\%-A^y-un$, and (b) linkage data of bp with a give a similar crossover value of 0.4% (Table 5).

The difficulty here is that these data put a^x practically on top of bp . If a^x is part of the A locus and is a mutation of the operator like A^s , then this puts A^s also exceedingly close to bp but three-point tests with bp , A^w and A^s although giving no definite answer, indicate that it is unlikely that bp lies between A^w and A^s or that A^s and bp are as close as a^x and bp appear to be (see RESULTS). One possibility is that bp lies 0.3% from the A complex as a whole and that crossing over within the locus is due to a different and independent mechanism from crossing over between loci. If one considers some intergenic crossover mechanism similar to that suggested by UHL (1965) then if intragenic crossing over occurred before the DNA double helices detach from their protein links, that is before intergenic crossing over occurs, it would have no effect on and be unaffected by any subsequent intergenic crossover.

The action of the A alleles can therefore be explained reasonably satisfactorily on the basis of an operon probably consisting of one cistron, where A^s and possibly a^x are mutations of the operon and the remaining alleles are structural in type.

No definite conclusion as to which, if any, of the above schemes is correct, can be drawn from the limited amount of data available; it can be assumed, however, that the A locus is an operon of one or more cistrons and that A^s is part of the locus, probably a mutation of the operator. Hence the use of the symbol A^s throughout, instead of As as the mutant was designated in Mouse News Letter (PHILLIPS 1963).

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SUMMARY

A gene A^s —agouti suppressor—is described, which shows 0.6% crossing over with A^w and a^t . As $A^s A^w/+a^t$ differs phenotypically from $A^s a^t/+A^w$, A^s is deemed to be part of the A locus, which forms an operon of one or more cistrons. The action of A^s seems to be similar to the effect of adding just sufficient sulphhydryl compounds to an *in vitro* system to allow only banded hairs to produce yellow pigment. There is still insufficient available evidence to decide whether or not the A locus consists of separate regions affecting back, belly, ears, etc.

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