A MUTATION OR A CROSSOVER IN THE HOUSE MOUSE ?

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I. INTRODUCTION

DURING the last fifty years there have been several reports of genetic activity at the agouti locus in the house mouse, *Mus musculus*. The phenotypic equivalence of some genotypes involving A^L (light-bellied agouti) and the genotype Aa^t (A is dark-bellied agouti and a^t is black-and-tan) led Pincus (1929) to suggest that not one but two loci may be the genetic basis of the apparent multiple-allelism so far observed. On this theory, one locus governs the belly colour and the other that of the back ; W * and w, representing white and not-white belly, and A and a, representing agouti and non-agouti back, are the symbols used by Keeler (1931).

Professor Sir Ronald Fisher, F.R.S., suggests that A^Y (yellow), because it differs from the other members of the series in several respects, *e.g.* in causing lethality when homozygous and in producing a tendency to obesity in heterozygotes, is due to a deletion covering more than one locus ; some little support for this idea is given by the fact that none of the mutations so far reported have occurred in matings involving A^Y. This factor will not therefore be considered in this paper in the treatment of the evidence for one or two loci as a basis of the agouti series.

Until now the events described have been interpretable, on either theory, only in terms of mutation. The occurrence of a possible crossover in the stocks in this department here reported, seems a suitable occasion on which to survey and discuss the evidence available on the genetic basis of the agouti series.

2. MUTATIONS OBSERVED IN THE LABORATORY

The genetical circumstances in which mutations have been observed have been somewhat varied, and the evidence for their occurrence is not uniformly conclusive. A brief account will facilitate discussion.
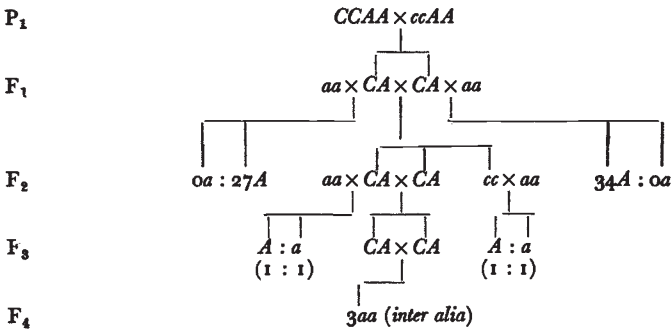
Table I gives the mutations reported by the various authors to whom Gruneberg (1952) refers in a section on the agouti series ; the case reported in this paper is added to the table (table 1).

* W, w used throughout this paper, does not refer to the locus at which dominant spotting occurs, which is normally given this symbol.

TABLE 1

List of " mutations " reported at the Agouti locus

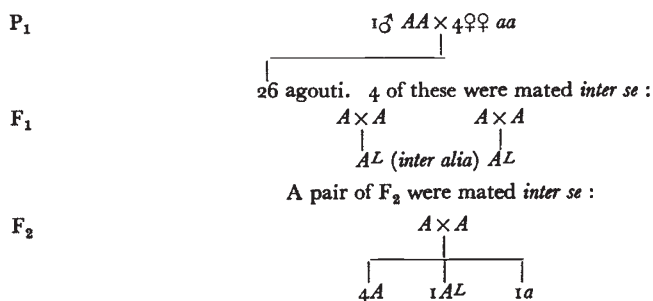| Year	Author	Number and genotypes of mutants	Parents of mutants
1908 } 1912 } 1916	Hagedoorn, A. L. Little, C. C.	2 Aa (in the same litter) 3 A^L (each from a different monogamous mating) 1 A^L	$AA \times AA$ $Aa \times Aa$ $Aa \times aa$
1929 1931	Pincus, G. Snell, G. D.	1 a^t 1 a^t } 1 a^t } each from a different 1 a^t } monogamous mating 2 a^t }	$aa \times aa$ $aa \times aa$ $Aa \times aa$ $Aa \times Aa$
1931 1947	Keeler, C. E. Little, C. C., and Hummel, K. P.	1 A^L 3 A^L (in different litters of the same mating)	$Aa \times a^t a$ $aa \times aa$
1949 1953	Bhat, N. R. Wallace, M. E. (reporting here)	1 A^L 1 aa	$aa \times aa$ $Aa^t \times aa$

Hagedoorn's 1912 report is an expansion of his earlier paper. His notation is different from that in current use and rather unwieldy, and he mentions contemporary misinterpretation. It is therefore with some diffidence that a simplified account is given here, although the material described is not complicated. In current notation, the phenotypic genealogy can be summarised :

P_1 $CCAA \times ccAA$

F_1 $aa \times CA \times CA \times aa$

F_2 $0a : 27A$ $aa \times CA \times CA$ $cc \times aa$ $34A : 0a$

F_3 $A : a$ $CA \times CA$ $A : a$
          $(1 : 1)$ $(1 : 1)$

F_4 $3aa$ *(inter alia)*

The genotype of both the F_1 mates, which were ancestors of the three black non-agoutis (aa), was proved to be AA, since they gave, in a testcross to non-agouti, 34 and 27 young each, all agouti. The descendants of the F_1 mates were also tested by crosses to non-agouti and one of the F_2 agoutis and the albino (cc) were found to be Aa. Hagedoorn gives no figures from the latter testcrosses, merely stating that the heterozygotes gave " equal numbers " of agoutis and non-agoutis, and that " the numbers of young produced from each test-mating always exceeded eight ". He concludes from these data that " this heterozygous nature of the two young produced from homozygous parents can only be explained by assuming that one of the parents had produced at least two gametes without G " (G is his symbol

for current A).   Since the two heterozygotes are stated to be from the same litter, it is possible that, if one parent produced two a gametes, the a genes could have arisen from the same or from separate mutations before or during gametogenesis, or that the parent was a gonadial mosaic.   It is also, of course, possible that both parents were involved, producing one a gamete each.   However, it is not easy to discriminate which explanation is the most likely, and it is sufficient for the purpose of this paper to show that the evidence for the occurrence of one, if not two, mutations A to a is strong.

Little (1916) describes clearly a large experiment, from which the relevant genealogy is here summarised :

P_1 $1\male\ AA \times 4\female\female\ aa$

                         26 agouti.   4 of these were mated *inter se* :

F_1 $A \times A$ $A \times A$

                      A^L (*inter alia*) A^L

               A pair of F_2 were mated *inter se* :

F_2 $A \times A$

                    $4A$ $1A^L$ $1a$

A fourth mutation to A^L occurred in a different experiment from a mating $Aa \times aa$.   That three of the mutants were closely related is clear since they all arose from the same stock within three generations of inbreeding.   Tests were made to establish (i) the allelism of the mutants (two of the mutants " have shown that their colour pattern was epistatic to grey-bellied agouti * and non-agouti "), and (ii) the identity with A^L of the fourth mutant which had a rather yellow belly. Little also states that the possibility of a mistaken identity

" is obviated by the fact that the F_2 generation white-bellied agoutis appeared in different cages at the Harvard Medical School where there had been no other white-bellied agoutis † for more than a year before the appearance of the mutant animals ".

Reassurance is thus given for the conclusion that four mutations to A^L occurred in Little's stock ; but it is not possible to distinguish whether the mutating genes were A or a.

Pincus' 1929 paper is a conclusive account of the occurrence of a mutation a to a^t.   A black-and-tan female, " No. 7048 " appeared

" in the ninth brother-sister generation of an inbred line of chinchilla non-agouti (black) piebald mice. . . . That this animal represented a mutation was apparent for the following reasons : (1) Its parents . . . produced in all 14 young in four litters, only one of which . . . showed the ventral coloration described ; (2) $\female 7048$, bred to totally unrelated non-agouti dark-bellied males, produced about equal numbers of white-bellied and dark-bellied young (20 light-bellied black and 10 dark-bellied black) ; (3) three of her sisters back-crossed to her father

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* Grey-bellied agouti is A.   † White-bellied agouti is A^L.

produced 16 young all dark-bellied ; (4) a brother and sister mated together produced 9 young, all dark-bellied. From these facts alone it will be seen that white belly represents a dominant mutation in one of the parental chromosomes so that a single animal heterozygous for the mutant factor appeared."

Pincus also satisfactorily eliminates the possibility of mistaken identities :

" First, no black and tan animals were in the laboratory at the time. . . . Second, the mutant resembled her litter mates in every respect."

Finally, he establishes the identity of his " white-bellies " with the black-and-tan described by Dunn (1928).

Snell (1931) reports the occurrence of four " probable mutations " to a^t, all born within three months of each other. The mutating gene in one case was certainly a since the parents were both aa, and in the other cases they may have been A or a : the parents of one were Aa and aa, and of the other two, both parents were Aa. Unfortunately two of the mutants died before breeding, and from the other two no breeding tests are reported. No indication is given of the relationship of the mutants, so that it is not possible to consider the independence or otherwise of these mutations. There is, however, some evidence, although necessarily inconclusive, that none of the mutants was in reality a black-and-tan transposed from other matings involving black-and-tans which did exist in the laboratory ; the genotypes of two of the mutants was in accord with expectation from their supposed parents. The four cases reported here can only be regarded, as Snell states, " as being, rather more probably than not, mutations to black and tan."

Keeler (1931) reports an unusual case. It can be summarised as follows :

P_1 $A \times a^t$

F_1 Several " Aat " were crossed to aa and one such mating gave :

F_2 $13aa$ $19A^L$
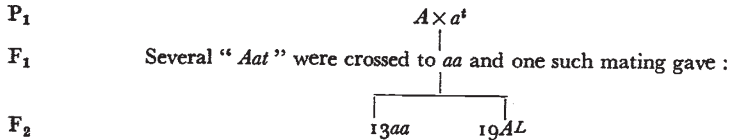
The paper does not state explicitly, but implies that the mates of the P_1 cross could have been Aa and a^ta. Keeler mentions the improbability of a crossover occurring in the zygote, " an unheard-of occurrence ", and concludes

" Hence it seems probable that a mutation from w to W occurred in the parental gamete furnished by the agouti parent, or what seems less likely a reverse mutation from a to A in the parental gamete furnished by the black-and-tan parent."

He does not consider the single-locus theory, on which the mutating genes may have been A, a^t or a. On the theory of two loci, the genealogy can be represented :
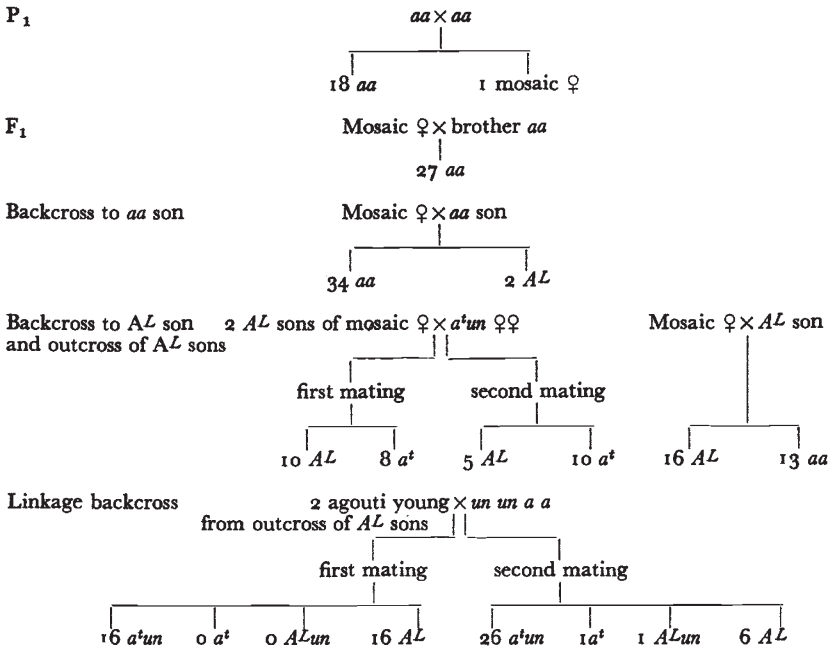
P_1 $\dfrac{Aw}{aw} \times \dfrac{aW}{aw}$

F_1 $\dfrac{AW}{aw}$

Little's (1947) report is the first case in which gonadial mosaicism is clearly indicated.

" The occurrence of three $A^W a$ * individuals in different litters from a single mating of the closely inbred dilute brown (*dba*) strain of mice, which has previously produced only *aa* animals since 1909, has been observed and is here recorded. The three individuals occurred among the progeny of ♀1 and ♂10, there being 18 normal young. . . . The frequency of the appearance of the mutant mice in the original mating suggests that one gonad of the mutating parent is a mosaic in respect to the formation of A^W and *a* gametes."

The mutation's frequency of appearance

" is sufficient to suggest a very early division of the gonad of the mutating parent into ' $A^W a$ ' and ' *aa* ' bearing cells."

No breeding tests of the A^W animals are reported. The possibility of an alternative explanation of the appearance of three closely related phenotypically identical mutants is, however, considered by

P_1

$$aa \times aa$$

18 *aa*   1 mosaic ♀

F_1

Mosaic ♀ × brother *aa*

27 *aa*

Backcross to *aa* son

Mosaic ♀ × *aa* son

34 *aa*   2 A^L

Backcross to A^L son   2 A^L sons of mosaic ♀ × $a^t un$ ♀♀   Mosaic ♀ × A^L son
and outcross of A^L sons

first mating   second mating

10 A^L 8 a^t 5 A^L 10 a^t 16 A^L 13 *aa*

Linkage backcross   2 agouti young × *un un a a*
from outcross of A^L sons

first mating   second mating

16 $a^t un$ 0 a^t 0 $A^L un$ 16 A^L 26 $a^t un$ 1 a^t 1 $A^L un$ 6 A^L

Little. He suggests that both parents producing the mutants were $+m$, producing $\frac{1}{4}mm$ which causes a mutation *a* to A^W in one of the pairs of chromosome V. This explanation is considered by him to be " somewhat improbable ". He states that an investigation is in progress ; but a private communication reports that an accident destroyed the material before any further progress was made.

Bhat's (1949) case is unique for this locus in that the mutant mouse was a mosaic somatically as well as gonadially ; thus the origin of

* A^W is alternative to the symbol A^L used throughout this paper.

the mutation is more certainly known than in most cases. He describes the appearance from non-agouti parents of " an altogether new type of animal possessed of irregular agouti-like markings on the back . . . an agout-non-agouti mosaic." The possibility of its arising from an agouti parent is eliminated on genetical grounds, and the allelism with the agouti series of the light-bellied agouti gametes it produced is established by a linkage test with un (undulated). Bhat concludes that " in all probability it was a reversion of the gene a to A^L or an equivalent allele." He does not exclude the possibility of its being " a mimic of A^L but closely linked to it," but continues that this " though not inadmissible, is too far fetched to be of any special significance." The breeding tests described by Bhat are summarised below : some additional data available in this Department have been added.

3. A POSSIBLE CROSSOVER (OBSERVED IN THE LABORATORY)

The first evidence of activity at the agouti locus which can be interpreted as a crossover (as well as on a mutational basis) is now given. In the stocks in this Department, a mating E692 gave the following performance :

$$\text{\Large\female}\ \frac{+}{py}\ \frac{B}{b}\ \frac{Sd\,fi\,a^t}{++A}\times\text{\Large\male}\ \frac{py}{py}\ \frac{B}{b}\ \frac{+fi\,a}{+fi\,a}$$

$$35\ A \qquad 1\ \text{\female}\ \frac{py}{py}\ B\ \frac{+fi\,a}{+fi\,a} \qquad 29\ a^t$$

(Sd is Danforth's short tail, fi is fidget and b is brown.)

Unfortunately the single aa mouse from this mating died while pregnant by an unrelated non-agouti male, a mating intended to establish allelism with the agouti series and identity of aa. That her mother was Aa^t and not A^La is evident from (i) her production of 35 A and 29 a^t young, and (ii) the fact that a dark-bellied agouti daughter and son produced, by unrelated non-agouti mates, respectively 3 A and 2 aa, and 4 A and 2 aa. The A gene of the mother was derived, via a mating $a^ta\times Aa$, direct from a stock in which there had never been any A^L.

It is very unlikely that the aa mouse was accidentally transposed from another mating, for the following reasons. Firstly, the aa mouse was also $fifi\ pypy\ B$, a genotype expected (excluding consideration of the agouti locus) with a fair frequency, about 1 in 20. Secondly, although there were other matings close by producing this genotype, this female appeared to be of the same age as her supposed litter-mates when the litter was classified for agouti, fidget and black, at 18 days old ; and the number and genotypes of the young in the cage then agreed with the number and genotypes observed when the litter was

first recorded at two days old and classified for sex, Sd and polydactyly. The very varied expression of polydactyly is fully described in these stocks, and it would be unusual to find many animals of the same age, sex and Sd genotype at any one time with the same degree of expression of this factor. Moreover, other factors, including W, p and d (dominant pied, pink-eyed dilution and Maltese dilution), not segregating in this mating, were segregating in most of the other matings in the stock.

On this evidence, it seems likely (i) that a mutation to a occurred from A or a^t, or (ii) that a crossover w/a occurred. This can be represented (using Keeler's notation), as follows :

$$\frac{Aw}{aW} \times \frac{aw}{aw}$$
$$\downarrow$$
$$\frac{aw}{aw}$$

4. MUTATIONS OBSERVED IN WILD POPULATIONS

There have been several reports of white-belly, made from observations on trapped wild specimens. The evidence on the genetic basis of the white-belly is not uniformly clear, and a short résumé would perhaps be useful.

TABLE 2

List of reports of white-bellied agouti observed in the wild

Year	Author	Location of wild population
1908	Morgan, T. H.	Massachusetts
1912	Dice, L. R.	California
1914	Clarke, W. E.	St Kilda Island, Scotland
1936	Elton, Ch.	Coalmine in Ayrshire, Scotland
1938	Philip, U.	
1946	Eaton, O. N., and Schwarz, E.	Virginia
1947	Falconer, D. S.	
1948	Engels, W. L.	Some North Carolina coastal islands
1949	Zimmermann, K.	Middle Europe

Morgan's (1908) report is very brief and gives no figures : " When the sport with a white belly is crossed with domesticated spotted mice the white-bellied character dominates." There is no indication of any breeding other than this initial outcrossing.

Dice (1912) is not concerned with the genetic basis of variation, but only with variation itself : accordingly, his account mentions no breeding tests at all :

" A considerable number of house mice in California have the under parts separated in color from the upper parts. The upper parts retain the color of the common house mice of the region, while the under parts become colored either white, creamy buff, reddish buff, or intermediate tints between these colors.

and the color of the underparts of the unmodified house mouse. In all, seven house mice with white underparts, two with creamy buff underparts and a much larger number with reddish buff under parts have been taken. . . . Enough intermediate stages between the various colors have been found so that it becomes certain that these grade into one another and therefore are probably the product of the same factor or factors of variation."

Engels (1948) referring to Clarke (1914) states " The mice of St Kilda Island were found to be exclusively white-bellied agouti, and hence presumably all homozygotes." Clarke's paper is purely descriptive, and uses no genetic terms or symbols.

Elton (1936) describes a colony of mice found in a coalmine in Ayrshire. Philip (1938) describes breeding experiments with 12 specimens, of which 6 were received directly from Elton and the others were taken from a colliery connected with the pit from which Elton took his mice. Six of the mice were yellow-bellied and

" when crossed with grey-bellied agoutis of the pure line (Strong CBA) gave only yellow-bellied offspring. Yellow-belly is thus shown to be a dominant and to have been present in homozygous form in the animals obtained. The backcross of the F_1 to the homozygous grey-bellied agouti gave 18 yellow-bellied and 21 grey-bellied offspring, showing that the difference was due to a single pair of allelomorphs. . . . Each animal was tested by 10 ; 12 ; 12 ; 13 ; 14 ; 16 offspring respectively."

—these numbers presumably refer to the six F_1 progenies from the six outcrosses. The wild dark-bellied mice were crossed with lighter agouti tame mice, and the outcrosses, and backcrosses and intercrosses therefrom gave various shades not easily assigned to a definite number of classes. The absence of heterozygotes for grey- and yellow-belly in her sample leads Philip to conclude that the mice in the coal pit " were not mating at random but break up into comparatively small breeding units."

Falconer (1947) shows that the " snowy-belly " found by Eaton and Schwartz (1946) in Virginia was probably not a new allele but an expression of A^L in the presence of modifiers. He establishes the identity of A^L by breeding tests.

Engels (1948) mentions only " six wild-caught white-bellied mice successfully tested ", from a total of thirty trapped. They were bred to wild grey-bellied mice, and apparently no laboratory animals were used at all. One white-bellied mouse produced no grey-bellies out of 20 offspring ; " six white-bellied mice "—it is not obvious whether these include the one just mentioned—" each with a grey-bellied mate, produced . . . 28 white-bellied and 19 grey-bellied. . . . Chi-square for the observed deviation from the 1 : 1 ratio is 1·72, a sufficiently satisfactory fit." There were, therefore, one homozygote and five (or six ?) heterozygotes, white-belly being thus proved dominant to grey-belly.

" Further evidence of the dominance of white-belly is afforded by two white-belly by white-belly matings involving one wild-caught mouse and three cage-bred mice of white-belly by grey-belly parentage."

These gave 9 white-bellies and 6 grey-bellies :

" Chi-square for the observed deviation from the expected 3 : 1 ratio is 1·80, a sufficiently satisfactory fit."

Engels concludes

" The above evidence identifies the gene involved as A^W, the dominant ' white-bellied agouti ' allelomorph of ' agouti ' . . ."

Zimmermann's 1949 paper is a survey of the distribution of various sub-species and variants of house mice. As such, it mentions no genetic tests, although it uses genetic symbols as if there had been genetic evidence for their use :

" Ein weiteres Kennzeichen von *domesticus* ist das Fehlen einer Demarkationslinie an den Flanken. Diese Färbung wird in der Genetik als ' Agouti ' (Symbol A) bezeichnet. Die ' Wildfärbung ' von *musculus* und *spicilegus*, bei der eine mehr oder weniger deutliche Trennung zwischen Oberseiten- und Bauchfärbung besteht, wird genetisch als ' Agouti weissbäuchig ' (Symbol A^W) bezeichnet, A^W ist dominant über A."

And later :

" Gelbes Pigment in den Distalhälften der Bauchaare tritt auch in Verbindung mit der A-Farbung bei *domesticus* auf. Bei deutschen *musculus* überwiegen die hellen Bauchfärbungen."

Some indication that A^W is in fact present in the feral sub-species is given by a mention of " snowy-belly " :

" Innerhalb der Wildfärbung bestehen für die Pigmentierung der Bauchhaare starke Schwankungen. Die Bauchhaare können völlig pigmentfrei sein und schneeweiss erscheinen (' snowy belly ', A^s), nach Eaton & Schwarz . . . ein weiteres Allel der Agouti-Serie, dominant über A^W. ' Schneeweisser Bauch ' ist vorherrschend bei Hausmäusen südlicher Trockengebiete, bei *musculus* und *spicilegus* tritt er nur als seltene Einzelmutante auf. (Im untersuchten Material 3 mal in Ostpreussen, i mal in Brandenburg, 1 mal unter 270 ungarischen *spicelegus*)."

Falconer (1947) suggests that, if in a population of AA with naturally selected modifiers for light bellies,

" a mutation from A to A^W took place, the major effect of this allele in combination with the minor lightening genes might well produce the extremely light phenotype seen in the snowy-bellied mouse."

The specimens investigated by Falconer certainly carried A^L : possibly, and for the same reason, those reported by Zimmermann also carried A^L. There cannot, however, be any certainty on this point, nor that the other " Agouti-weissbauchig " mice reported by Zimmermann also carried it.

5. DISCUSSION

Several cases of multiple-allelism have, of recent years, shown themselves to be, more probably, cases of close linkage—the Rhesus factor in Man being the classic example. An analysis of the evidence for these two genetic situations in a particular species is therefore of some value, and it is hoped that some of the arguments developed here may be of use in similar cases in other species.

In order to arrive at some conclusion, however tentative, as to the number and kinds of activity that have occurred at the agouti locus, it is necessary to examine the evidence and to reduce it to a small core about which there is some degree of certainty.

In a survey of this kind, each case must be judged on the material reported. Inevitably, some omissions and ambiguities in the reports must be pointed out, and possible explanations, other than those given by the authors, must be stated whatever their implications. It must be emphasised that the primary aim of this paper is not a criticism of technique, but an evaluation of the evidence reported.

First, a routine point which, if elaborated by the author, strengthens the claim for mutation, is that of identity of the anomalous animal. Not all the reports give assurance in this respect. For example, evidence in Keeler's (1931) paper against the possibility of mistaken identity would have been welcome—perhaps more so in this case than in others. For here the anomalous mouse was of the same phenotype as its supposed litter-mates : only its breeding behaviour distinguished it. It is unfortunate that no evidence from other segregating factors was available to give assurance as to its parentage. However, the fact that Keeler does not mention A^L as present in his laboratory may perhaps be taken as circumstantial evidence in favour of mutation.

It would seem to be of importance that, in many cases, breeding tests were not made with the mutants. Their resemblance to a member of the agouti series is often taken as an indication of their identity with that member. Proof of identity has, empirically, three parts : the establishment of (i) heritability of the factor, (ii) identification of its locus, usually by allelomorphism with another factor at the locus, or by linkage with a neighbouring factor, and (iii) identity with a particular allele.

In cases where the expression of a gene is very variable, identity tests are particularly desirable. There is much published evidence of the variability of members of the agouti series, particularly as regards the belly colour (Little, 1916 ; Pincus, 1929 ; Schwarz and Schwarz, 1943 ; Kaliss, 1942 ; Falconer, 1947 ; Zimmermann, 1949 ; etc.). Thus, some of the reports of a^t and A^L, occurring both in the laboratory and in the wild, omit description of identity tests or describe crosses made for other purposes, and they thus leave open the possibility of a polygenic explanation of the observations made. The examples whch follow are largely taken from reports of mutations in the wild where no knowledge of multi-factorial genotype is available until breeding tests are made, a circumstance which does not prevail to the same extent in the laboratory.

In Engel's (1948) reference to Clarke (1914), the word " presumably " seems to imply that genetic tests were not done to test the suggestion that all the mice of St Kilda were $A^L A^L$, and, in fact, Clarke's report does not mention breeding tests, being concerned

merely with descriptions of specimens. An advantage for white belly on this island may equally have resulted in a natural selection for factors lightening the expression of AA. From Dice's (1912) report there is little alternative to the conclusion that the variation he observed was genetically multi-factorial. Yet Gruneberg (1952) includes references to Clarke and Dice in a context which rather easily allows the interpretation that theirs are reports of mutations to A^L :

" That mutations from A to A^W are possible follows from the occurrence of sporadic light-bellied mice in dark-bellied populations (*e.g.* cases reported by Morgan, 1908 ; Dice, 1912 ; Elton, 1936 ; Philip, 1938 ; Engels, 1948 ; Zimmermann, 1949). . . . It thus seems that mutations at the agouti locus are common both in the wild and in the laboratory. . . . There are even whole populations, such as that of the island of St Kilda (Clarke, 1914) in which A^W has completely replaced A^+."

A further statement by the same authority is somewhat misleading :

" According to Schwarz and Schwarz (1943), all feral sub-species of *M. musculus* are A^W, and the dark belly of A^+ is the hall-mark of commensalism. However that may be, it is clear that A^W and A^+ can be regarded as wild-type alleles of this species with equal justification."

Schwarz and Schwarz do not mention any breeding tests, but they do state :

" Specialisation in all commensals proceeds along the same lines. The wild forms are white-bellied and short-tailed, the most specialised commensals grey-bellied and long-tailed. There are intermediate types corresponding to the stage of commensalism."

It is difficult to make any certain conclusion from this, or from Zimmermann's paper, as to the incidence of A^L in the wild. These two papers, and those of Dice and Clarke, do, however, indicate that some environments favour a light-belly, and one can conjecture —but it is no more than a conjecture—that mutation to A^L could easily spread and even become homozygous in some populations. Falconer's (1947) suggestion about the origin of " snowy-belly " and Zimmermann's account of several snowy-belly mice caught in the wild, are some support to the view that mutations to A^L are common in the wild ; but it appears that no certain conclusion can be drawn until more genetic tests have been made with wild specimens. On the present evidence, considering only those cases in which such tests have been described, there appear to be only three distinct mutations reported—Philip's, 1938 ; Engel's, 1948 ; and Falconer's 1947.

A further point on which fuller information would in some cases have been useful, is the relationship of apparently distinct mutations occurring at about the same time in the same laboratory. In Little's (1947) case, it is clear from the account that only one event affecting the agouti locus is necessary to account for the appearance of three " mutants ", *viz.* gonadial mosaicism in one parent. In Hagedoorn's (1908) and Little's (1916) reports, close relationship is described, a

circumstance which suggests the possibility of some genetic cause
(other than mosaicism, at least in Little's case) for what has otherwise
to be regarded as a very rare coincidence. Little satisfies himself
that the recurrence of mutations to A^L were not due to " inbreeding,
hybridisation, selection." He describes a second inbreeding experi-
ment parallel with the first, from which the mutants were obtained,
and states that after three generations and the scoring of 4500 mice,
" none of these animals were white-bellied agoutis." This experiment
disposed of explanations which were possibilities according to con-
temporary knowledge ; but it does not exclude the presence of a
factor in the first experiment, absent from the second, which increases
the mutation-rate at the agouti locus. Doubtless other explanations
could be conceived if further evidence were available. That no
details about relationship are given in Snell's (1931) account, is
unfortunate since he reports the greatest number (four) of simultaneous
mutations, all to the same allelomorph.

Although the phenotypic equivalence of A^L and Aa^t has been
known at least since 1929 (Pincus), the necessity for establishing the
identity of the factor causing light-belly in agoutis has not always
been realised. Intercrosses of light-bellied animals (Engel, 1948) or
crosses involving a (Little, 1916) were apparently an unconscious
safeguard in some cases ; the fact that black-and-tan is not reported
as appearing from such crosses is some reassurance that the mutations
were in fact to A^L and not to a^t. (It should perhaps be pointed out,
however, that although from Engel's intercross 9 white-bellies to
6 grey-bellies is not a bad 3 : 1, this ratio is not a much worse approxi-
mation to the 1 grey-belly : 2 white-bellies : 1 black-and-tan expected
if his cross was $Aa^t \times Aa^t$ and not $A^L A \times A^L A$.) No mention of the
possibility of a mutation to a^t is made in any of the reports of light-
bellied agouti observed in the wild. In some cases, as for instance
the evidence for homozygosity of light-belly in Philip's (1938) and in
Engel's (1948) reports, there are in fact good grounds for believing
that the mutating genes were in fact A^L and not a^t. In general, however,
the absence of evidence for the presence of a^t rather than a definite
report of the absence of a^t has to be taken as indicating mutations to
A^L in the wild. Mutations to a^t, if they occurred, while producing
light-bellies, would not become as widespread in wild stocks as those
to A^L, since only the heterozygotes would have the advantageous belly
colour. But it needs to be borne in mind, when studying wild popula-
tions, that sporadic mutations to a^t may be picked up, and that there
may even be balanced populations of three genotypes with Aa^t the
most common.

A final comment on the evidence must be made. It is striking that
in all the reports of multiple mutations the mutations are always to
the same gene. This seems an unusual coincidence, but it is doubtful
whether there is any satisfactory explanation. Possibly it comes within
human error : the observer, having noticed one mutation, tends to

interpret rather uncritically as similar mutations other anomalies which may have simpler explanations such as mistaken identity, multifactorial variation, etc. This argument reduces the estimate of the number of real mutations. On the other hand, there may have occurred more mutations than have been reported, but the observer, having seen one, tends to notice only similar rather than dissimilar ones. As it is impossible to discover the correct explanation, a conservative estimate of the number of real mutations will be made on the assumption that some human error probably has occurred, and that it has occurred more often in a direction which increases the apparent number than in the opposite direction.

To sum up : possible explanations of the unexpectedly large number of simultaneous mutations are as follows. Firstly, non-genetic causes :—mistaken identities ; mistaken interpretation of observation ; selective observation. Further, there may be some environmental agency, which stimulates a particular gene to mutate in a particular direction. This latter cause is not generally accepted, but it seems to have some reasonableness from the present evidence. Secondly, genetic causes : gonadial mosaicism (somatic or not) ; and a factor or factors increasing the mutation rate at the agouti locus.

With these possibilities in mind, and with the reservations indicated in the preceding paragraphs, some idea of the minimum number of events at the agouti locus may now be obtained (table 3).

Even when reduced to a minimum, the number of mutations to light-belly (whether or not the dorsal colouration is agouti or non-agouti) far exceeds the number of any other kind of mutation observed in the laboratory or in the wild.

Mutations to light-belly are far easier to observe in the wild than are those to dark-belly, since a greater number of populations of dark-bellies than of light-bellies are known ; also, mutations to non-agouti (a on both theories) are difficult to observe because they are not distinct from the " wild " phenotype unless homozygous. But it is doubtful whether these considerations can appreciably alter the excess of mutations involving light-belly observed in the laboratory. Some degree of inbreeding is practised in a great number of experiments, and this process should reveal recessive mutations in stocks homozygous for any member of the series except a. Moreover, no mutation to dark-bellied agouti has been reported despite the fact that a large number of stocks are kept for the study or preservation of coat-colour mutants, and are run with members of the agouti series which are not agouti dorsally ; whereas at least two mutations to light-bellied agouti have been reported as arising from stocks homozygous for non-agouti, in which mutations to dark-bellied agouti would be equally striking.

The real mutation-rate of each member of the series, and a final decision as to whether it is based on multiple allelism or close linkage,

cannot be obtained without a very large-scale experiment ; such an experiment is not likely to be made, except perhaps incidentally in stocks conserving compatible factors, since the solution of this problem is at present not worth the labour this would require.   At present, therefore, conclusions have to be drawn from data which may have a slight bias in favour of one mutation rather than of another.   However, as there is no *a priori* reason for thinking the data biased, a tentative conclusion can be made that light-belly is more stable than dark.

TABLE 3

A conservative estimate of the number of mutations at the agouti locus

Year	Author	Number and identity of mutating genes on basis of	
		One locus	Two loci
A. *Observed in the laboratory*			
1908 } 1912 }	Hagedoorn	1 A to a	1 A to a
1916	Little	1 A or a to A^L	1 w to $W \pm a$ to A
1929	Pincus	1 a to a^t	1 w to W
1931	Snell	1 A or a to a^t	1 w to $W \pm A$ to a
1931	Keeler	1 A, a or a^t to A^L	1 w to W or a to A
1947	Little	1 a to A^L	1 w to $W + a$ to A
1949	Bhat	1 a to A^L	1 w to $W + a$ to A
1953	Wallace (this paper)	1 A or a^t to a	1 W to w or A to a or a crossover in $\frac{A\,w}{a\,W}$ giving aw
B. *Observed in the wild*			
1938	Philip	A to A^L	w to W (in a coalmine in Ayrshire)
1948	Engels	A to A^L	w to W (some N. Caroline coastal islands)
1947	Falconer	A to A^L	w to W (via Eaton and Schwarz from Virginia)

If this is so, and if there is only one locus, two alleles (A^L and a^t) must be more stable than the other two.   Whereas, if there are two loci, theory requires only one allele (W) to be more stable than its single counterpart.   If it is admitted that " top-stability " is more likely to be confined to one rather than to two alleles, the theory of two loci has slightly more probability.

Incidentally, on the data there is no reason to suppose that dorsal agouti is more stable than non-agouti, although in general there have been slightly more mutations in this than in the opposite direction. If it is conceded on the theory of two loci that the loci are so close that a mutating agent affecting one would frequently affect the other simultaneously, then the two observed mutations a to A^L agree well with this theory.   On the other hand, if there is only one locus, they

must be considered as mutations involving " two steps up at once " (a, via one of the two dominance-equivalents, A and a^t, to A^L), an event which future data may reveal to be more rare than simultaneous mutations of adjacent loci. To resume, on the basis of stability of light-belly, then, the most likely explanation appears to be that there are two loci, and that the allele W, and perhaps also the allele A, show relatively high stability.

In every case except the first a mutation " down " the series, that is, in the direction dominant to recessive, is avoidable on either theory. In the 1953 case it is avoidable only if the case is considered as a crossover. At present there is no method by which a lower limit can be set to recombination frequencies, nor an upper limit to " down " mutations. Their ranges may, in fact, overlap. Distinction must be made on other grounds than the theoretical frequencies of each. Nevertheless, it is usually safer to interpret a single rare event as a crossover rather than as a " down " mutation, since the general frequencies of the former have a much higher range than those of the latter. On the basis of the direction of mutation, then, the theory of two loci again has a slight advantage.

Finally, on evolutionary considerations, it appears that the theory of two loci has more probability than the other. It is difficult to understand why the two alternative genotypes on the theory of one locus, Aa^t and A^L, should have, as a result of selection, the same phenotype : if the two phenotypes have been in competition, it is to be expected that the one which can become homozygous genotypically, and maintain the same phenotype, would have an advantage over the one which can only produce two homozygotes different in phenotype from itself and rather strongly different from each other. Selection does not usually favour quite such a large degree of adaptability to new surroundings as is thus shown by the genotype Aa^t, and in fact there is no certain case of polymorphism of AA, Aa^t, a^ta^t known in the wild. In the knowledge that there are now environments which favour light-belly as well as dark, but that there are none favouring non-agouti dorsally, and assuming an almost equal mutation-rate for all members of the series, one would expect selection to result in almost equal dominance of A and A^L, and complete dominance of these two over a^t and a. (Incidentally, if more were known about the factors modifying A^L and A in the wild, it would probably be found that selection can produce interchangeable dominance between A and A^L.) On the theory of two loci with more or less independence of the direction of mutation at each locus, selection could work differentially on the back and the belly, and there would result the dominance interactions now observed : A is now dominant to a—in terms of this theory—and W to w, dominance of W being conditioned to some extent by the allele at the neighbouring locus * and modified by cumulative factors. And there

* The classification of the belly is easier in the presence of a than of A.

is no inconsistency in the phenotypic equivalence of the genotypes AW/aw and aW/Aw, these being the double heterozygotes in coupling and in repulsion respectively.

6. SUMMARY

1. A brief account is given of previous reports of mutations observed in the laboratory.

2. A new event at the agouti locus is described, which, unlike previous events, can be interpreted either on a mutational basis or as a crossover.

3. A brief account is then given of previous reports of light-belly observed in wild populations.

4. A discussion follows. An attempt is made to evaluate the evidence so far available on the number and kinds of activity which have occurred at the agouti locus.

5. From this it appears that there is much suggestive material, but not much real evidence, for the belief that A^L is common in the wild and that consequently it can be regarded as a " wild-type " along with A with equal justification. More genetical tests must be made before such an assertion, which has much theoretical probability, can be made with any certainty.

6. From all the reports, a conservative estimate is made of the number of real mutations that have occurred. From this estimate, and from entirely theoretical considerations, arguments are put forward which indicate that the theory of two loci has slightly more in its favour than the single-locus theory. This appraisement is intended not so much in order to arrive at a final and certain conclusion—which is impossible on the present evidence—but in order to show what still needs to be done before a certain conclusion can be made.

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