ANALYSIS OF A CASE OF MOSAICISM IN THE HOUSE-MOUSE.

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(With Plate XXIII.)

Mammals with mosaic coat patterns in which contiguous areas show differently coloured hairs occur very rarely, but the analysis of such cases is of importance for interpretations of the effects of the genes on development. One such case which has occurred in a laboratory stock of house-mice is being studied in detail, and some conclusions of interest are already apparent.

The original animal showing mosaic coat colour was a black male which was spotted with small areas of light tan on the back and head (Pl. XXIII). The spots comprised about one-tenth of the dorsal surface. None were found on the lower surface. This animal was the offspring of a cross of a black-and-white variegated male by a yellow female, both parents being members of families inbred for eleven and for five generations respectively. The yellow parent was known to be $A^yaBBC^+c^rWwSS$ and the black parent $aaBBC^+C^+SsWw$[2]. The black mosaic son was thus supposed to be either a black self ($aaBBC^+c^rS$–ww) in which **a** had mutated to A^y in a cell ancestral to the light-coloured areas, or a yellow (A^ya) in which A^y had been lost from all skin cells except those in the light areas. However, when tested by non-yellow mice (blacks and agoutis) he produced no yellows out of ninety-eight offspring, indicating that his gonads were **aa**. The same tests showed that he was genetically **ww**, *i.e.* he did not carry the **W** white spotting gene.

The mosaic was also out-crossed to homozygous wild-type stock (black agouti) to test whether he contained a new mutation for mosaic coat colour such as is found in black and red guinea-pigs. The F_1 animals (ten) were normal wild-type colour. An F_2 of thirty-six animals contained no mosaics. An out-cross to a standard yellow stock (A^yA^1BB) gave three yellows, five agoutis in F_1 and twenty yellows and ten agoutis or

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[2] A^y yellow; **a**, non-yellow; **B**, black; C^+, full colour (wild type); c^r, ruby dilution—allelomorph of c^a (albinism); **W**, variegated white spotting; **s**, piebald white spotting; A^y and **W** are lethal when homozygous.

blacks in F_2 but no mosaics. When bred to his black mother he produced nine blacks including no mosaics. When bred to three daughters he produced fifteen coloured animals including no mosaics.

By one black female which was C^+c^r he produced one sepia offspring which resembled the phenotype of c^rc^a. This animal, when bred, did indeed segregate for c^r and albino. Since both parent stocks of the mosaic were known not to carry albinism, this must have arisen *de novo* in the mosaic. Re-examination of the light areas of the mosaic suggested that they might be c^rc^a or c^rc^-.[1] The hairs in these areas were lighter than c^rc^a as this genotype is ordinarily expressed in black animals, and it was assumed that absence (deficiency) of the colour locus in these areas might produce a lighter colour than c^rc^a. The hypothesis which subsequent experiments were designed to test was that the mosaic had begun development as C^+c^r and that the wild type gene (C^+) had been lost from certain areas, leaving them c^-c^r. If the event which led to this change had affected the gonads, as well as certain somatic tissues, it might be expected that the mosaic male would produce gametes containing C^+, c^r, or c^-. Since c^- produces effects similar to albinism, he should thus appear to segregate for three members of the same series of multiple allelomorphs.

Such proved to be the case. When crossed with albinos (c^ac^a), ruby dilutes (c^rc^r) and extreme dilutes (c^dc^d) the mosaic male produced the following progeny:

Test females	C^+	c^rc^a	c^rc^r	c^rc^d	c^-c^a	c^-c^r	c^-c^d
Albino c^ac^a	22	25			5		
Ruby dilute c^rc^r	17		20			8	
Extreme dilute c^dc^d	19			26			4
Tested gametes of mosaic male	58 C^+		71 c^r			17 c^-	
Tested F_1's from out-cross to C^+C^+	3		5			1	
Total (from adult descriptions)	61		76			18	
% (from adult descriptions)	39·3		49·0			11·6	
*Total (including descriptions at birth)		170				20	
% (including descriptions at birth)		89·5				10·5	

* Several litters sired by the mosaic male on albino mothers were killed at birth, when the identification of c^- offspring could be made with certainty. The c^+ and c^r offspring could not be distinguished with the same accuracy. The data are included to give a better estimate of the frequency of c^- gametes.

It is apparent that the mosaic produced gametes with three conditions of the **c** locus. These results mean that the gonad of this male is also mosaic for the locus involved. If the mosaicism of his skin and gonad

[1] The albinism arising from the mosaic is hereafter designated as c^-, since it appears to be either a non-lethal deficiency at the **c** locus or a new albino gene.

arose from the same change, this probably occurred in a cell or cells ancestral to certain areas of both the epidermis and the gonad. The cell lineage of these tissues can trace to a common source only in very early stages of embryogeny.

One indication of the nature of the change is suggested by the fact that nearly the normal proportion (50 per cent.) of c^r gametes was produced, while the C^+ gametes were deficient in frequency by an amount (10 per cent.) which corresponds roughly to the frequency (11 per cent.) of the new albino (c^-) gametes. The c^r gametes comprise about half of the total, and the sum of the C^+ and c^- gametes makes up the other half. The changed condition of the c locus thus probably arose in a C^+ chromosome. Of the expected 50 per cent. of C^+ gametes about four-fifths were actually C^+ and about one-fifth c^-. From this we may assume that the change from C^+ to c^- occurred in a pregonial cell from which about one-fifth of the gonad was descended. Four-fifths of the gonial cells would then be normal C^+c^r and produce at reduction equal numbers of C^+ and c^r gametes; one-fifth would be c^-c^r and produce equal numbers of c^- and c^r gametes. The final distribution of gametes under these conditions would be 40·0 per cent. C^+, 50·0 per cent. c^r, 10·0 per cent. c^-, as obtained. The change in the C^+ chromosome might be (1) the loss of the entire chromosome, (2) loss of a portion bearing C^+, or (3) mutation of C^+ to c^-. Translocation rather than complete loss of a fragment with the C^+ locus should leave a deficiency in the c^- chromosome, and should thus give the same effect as hypothesis (2). To distinguish among these possibilities the c^rc^- and c^dc^- progeny of the mosaic were tested in various ways.

If the entire C^+ chromosome were deficient in the c^- gametes, then other recessive genes in the chromosome pair bearing the c locus should show pseudodominance when combined with c^-. Two recessive genes have been located in this chromosome: sh^l, shaker (Gates, 1931), which shows about 3 per cent. crossing-over with the c locus and is consequently very close to it; and p (pink-eyed coloured) which is more distant, at about fifteen cross-over units. Animals of genotype c^rc^- and c^dc^-, offspring of the mosaic, have been crossed with $c^ac^ash^lsh^l$ and with c^dc^dpp. Neither sh^l nor p shows pseudodominance in the albino and c^dc^- progeny respectively, indicating that the wild-type allelomorphs of these genes are present, and that c^- is due neither to absence of a chromosome nor to a deficiency at the c locus extensive enough to include the locus of sh^l.

On the other hand the deficiency might extend from c in the opposite

direction from sh^1, and this region has no loci marked. Any extensive deficiency, however, might be expected to be lethal when homozygous, and thus c^rc^- (or c^dc^-) when bred to c^rc^- (or c^dc^-) should produce no albino c^-c^-, although both c^rc^- and c^dc^- should produce some albinos (c^ac^-) when bred to ordinary albinos (c^ac^a). Several tests of both sorts have been made. The present figures from all *inter se* matings of animals heterozygous for c^- are 183 c^r or c^d : 55 c^-c^- (expected 178·5 c^r or c^d : 59·5 c^-c^-); matings of c^rc^-, c^+c^- and c^dc^- by c^-c^- have given 29 c^nc^- : 23 c^-c^-. The c^-c^- albinos thus appear to be of normal viability and fertility and it is therefore unlikely that the change to c^- involves any extensive deficiency. c^rc^- and c^dc^- mated to albinos have produced 26 c^rc^a or c^dc^a : 18 albinos (c^ac^-), a normal segregation for c^-. Matings of c^rc^- by c^rc^r have given 10 c^rc^- : 13 c^rc^r, again showing that c^rc^- offspring of the mosaic segregate as though they contained an albino allelomorph. These tests appear also to dispose of the assumption that the C^+ locus has undergone translocation to another chromosome, since in that case the c^- chromosome (from which C^+ had been translocated) should show a deficiency. Moreover, if the c^rc^- patches in the mosaic were due to loss of an unstable C^+ translocation in somatic mitosis, the transmission of the translocation might be expected to result in further mosaics among the F_2 progeny of the mosaic. None has been found.

In addition to random matings among F_1 animals from out-crosses of the mosaic to wild type, which would be sufficient to test a monofactorial segregation of a gene for mosaicism, F_1 animals of genotype C^+c^r and C^+c^d have been bred to F_1 c^rc^- or c^dc^-. 108 F_2 progeny from these matings have been observed, but none was mosaic. This type of mating brings together the three conditions of the albino locus which were present in the mosaic, and presents the maximum opportunity for the development of mosaicism if it is due to unstable translocation or to other hereditary influences. That it has never been found among the descendants argues against the transmissibility of the condition, and supports the interpretation that mosaicism in the original animal was due to a mutation affecting both gonad and skin.

The experiments thus far have shown that the gene C^+ was not present in about a fifth of the gametes which should have received this chromosome, that the change did not involve deficiency for this chromosome or for any extensive part of it, and that zygotes homozygous for the changed chromosome (c^-c^-) are viable. The change at the C^+ locus is thus either a new mutation to albinism (c^a), or to a new allelomorph of albinism (c^-), the effects of which in homozygous form are

indistinguishable from those of c^a; or else c^- is a deficiency too small to be lethal. If either of the latter assumptions is the correct one, the diluting effects of c^- in compound with c^r or c^d might prove to be different from those of c^a and thus provide a criterion for distinguishing c^- from c^a. It has been found that the c^rc^- offspring from the mosaic and as derived from the above crosses resemble in general the c^rc^a compounds in colour. When combined with black (**aaBB**) the colour of both compounds is sepia brown, easily distinguishable from the dull black of c^rc^r. To test minor differences in effect of c^a and c^-, c^ac^- albinos have been crossed with c^rc^r. If c^a and c^- are the same, only one class of progeny should be produced, *i.e.* sepia compounds like c^rc^a. Actually, however, two classes of progeny have been obtained in some crosses, *i.e.* normal sepia like c^rc^a and a lighter sepia which may be c^rc^-. Much more extensive experiments will be required to test whether this difference is constantly associated with c^-, since other modifying genes affecting the grade of colour must be made uniform by continued back-crossing of c^rc^a and c^rc^- to a pure c^rc^r stock. The difference noted, however, agrees with the observation that the c^rc^- areas in the mosaic are lighter in colour than c^rc^a. As a working hypothesis we may therefore assume that C^+ mutated to c^- in a cell ancestral to parts of the skin and the gonad, and that c^- is probably an allelomorph of the albino series, with effects below those of c^a. Although an exactly analogous situation has not been noted, Timoffeef-Ressovsky has recently reported two mutations to white-eye in *Drosophila*, in which the phenotypic effects of the gene are slightly different from those associated with previous mutations to white. White-eye, like albinism, has occupied the lowest position in a series of multiple allelomorphs, but it is reasonable to expect that further mutations in the same direction should produce allelomorphs with effects below the threshold of visible change in the chief character affected, and that such changes should be detectable only in compounds with the new allelomorph.

The mosaic male was also tested for spotting genes, since both parents were heterozygous for **W** which, under certain conditions of heterozygosity, leads to variegated or black-eyed white spotting (**WW** is anaemic and lethal), while one parent was heterozygous for **s** which, when homozygous, leads to piebald spotting. By self-coloured animals from pure **wwSS** stocks, the mosaic male produced 164 self-coloured offspring; by variegated (**WwSS**) mates he produced thirteen variegated and eight self-coloured and no anaemics. These results show that he does not transmit **W** and is thus **ww**. By piebald (**wwss**) he produced two

Ss animals; by three self-coloured daughters (**wwSs**) he produced fourteen selfs and five piebalds. Crosses *inter se* of eight of his F_1 self-coloured offspring (from cross of mosaic by self) gave twenty-seven selfs and twelve piebalds. Tests of such F_2 piebalds by unrelated piebald and *inter se* gave only piebalds. These results show that the mosaic male was heterozygous for the piebald gene **s**. His genotype for the spotting genes tested was **wwSs**. The tests showing him to be genetically self-coloured, but heterozygous for recessive piebald are of importance in view of the frequent association of mosaicism and spotting which is discussed below.

Discussion.

A black mouse which showed several areas of tan-coloured hairs has been shown to segregate for three members of the albino series of allelomorphs. He produced gametes in the proportion ·4 $\mathbf{c^r}$: ·5 $\mathbf{C^+}$: ·1 $\mathbf{c^-}$, indicating that the fertilised egg from which he arose was $\mathbf{C^+c^r}$ and that $\mathbf{C^+}$ mutated to $\mathbf{c^-}$ in a cell from which about one-fifth of the gonad was derived. Since several patches of skin showed a colour associated with the phenotype $\mathbf{c^rc^-}$, it is highly probable that the mutation occurred in a cell which gave rise both to tissues which became included in the gonad and to tissues which in later development came to form isolated portions of the skin. Mutations at the albino locus have been so extremely rare in mice as to make it very unlikely that the somatic variation in skin colour and the mosaic condition of the gonad originated in separate mutations. An interpretation in the same terms of mosaicism in these two different tissues requires the assumption that the cell lineages of skin and gonad converge in development at a period early enough to permit the same changed cell to give rise to both. It is even more significant that *only a fraction* of each tissue shows the same change, that is, that about four-fifths of the gonad "anlage" was formed before the change occurred, and that nearly all the cells ancestral to the epidermis had received the unmutated gene.

Still further problems are raised by the peculiar distribution of the mosaic patches in the skin. These occur in widely separated parts (Pl. XXIII) on the head, the neck and in the lumbar region of the back. The occurrence of somatic mosaics in animals generally in which well-known colour genes are shown to be distributed differently to different areas (see below) indicates that the colour reaction of a cell is governed chiefly by its own genes. This conclusion has been drawn by Wright (1926) from an examination of mammalian mosaics, and it is clearly apparent from the studies of mosaics and gynanders in *Drosophila* by Morgan, Bridges,

Sturtevant, Muller, Mohr and others. Danforth has shown by transplantation experiments with feather follicles that it is strictly true for the colour and pattern of the feather, although general endocrine agencies may modify the reaction within narrow limits. This being the case, we are probably justified in inferring that the changed colour in the mosaic patches is due to the presence of the changed locus in each of these patches, and that this changed locus was received from the original cell in which the change occurred. This means that the patches which later in development became separated by areas of normal tissue were in earlier stages united, indicating a complex series of differential growth rates or migrations in different directions of the tissues which compose the epidermis from which the hair follicles and the pigment cells are derived. Although such a viewpoint may appear to increase the difficulties of interpreting the distribution of coloured and white areas in white spotted animals, in which all the cells may have the same genetic constitution, it does provide a basis for the assumption of heterogeneity in the epidermal tissues, due to the different growth rates which may obtain in the developmental history of different areas. It may be pointed out incidentally that in the present case the dorsal areas showing the new or changed colour are those which are most often white in piebald or variegated spotted mice. On the other hand, the area most frequently white in all spotted mice is the belly, which in this case shows no mosaic patches.

Comparison with other cases.

Wright (1926) has reviewed the genetically analysed cases of vertebrate colour mosaics reported up to 1926. He has pointed out the different mechanisms involved. Somatic mutation or somatic loss of a dominant gene from a heterozygote is the most frequent. Only one case involved mutation affecting both soma and germ cells. This case, analysed by Wright, is most nearly analogous to the case described above. This concerns an agouti guinea-pig with areas of intense (C^+) and dilute (c^dc^d) colour. This animal also bred as a germinal mosaic in which 70 per cent. of the germinal epithelium was C^+c^d and 30 per cent. c^dc^d, based on 228 offspring. Wright assumed that the animal began development as c^dc^d and that c^d mutated to C^+ in a cell ancestral to parts of the gonad and to two areas of the skin. He has also pointed out that in three of his mosaics and in a tricolour mosaic described by Castle (1922) the mutant areas are scattered, showing that a single cell can give rise to scattered descendants. In none of these cases was mosaicism as such

found to be inherited, although the relationships among Wright's seven mosaics were closer than would be expected to occur by chance, and he thought this might indicate an hereditary tendency toward germinal instability.

Since 1926 several additional cases of mosaicism have been reported[1]. Four[2] of these have occurred in the mouse (Fisher (1930), one case; Pincus (1929), three cases) and all were black spotted animals heterozygous for brown (**Bb**), which showed each a single brown area. These may all be interpreted as due to loss of **B** from a somatic cell and its descendants. Pincus, however, suggested that since the brown areas occurred in regions which are occasionally white in piebald mice, the recessive gene **b** may exercise a controlling effect in such areas. A similar association of mosaic areas with white spotted areas has appeared in several intense-dilute mosaics in rabbits described by Castle (1929).

A survey of the twenty-one cases of rodent colour mosaics described in the literature shows that although seven different loci are involved all except two or possibly three of the cases have occurred in white-spotted animals. The two certain exceptions are Wright's germinal-somatic mosaic guinea-pig, and the germinal-somatic mosaic mouse described in this paper, which showed no white spotting although it was heterozygous for a recessive spotting gene. Both of these occurred in non-spotted animals, and both almost certainly involve new mutations. The doubtful exception is Castle's (1912) mosaic guinea-pig which probably began as $\mathbf{c^d c^a}$ and lost $\mathbf{c^d}$ from large areas of the coat, but of which the spotting

[1] In addition to the published cases one occurred in our laboratory in 1929. This was a black "Dutch-marked" mouse (piebald spotted) which showed on one shoulder a small patch (1 cm.²) of blue-grey fur resembling the blue dilute or Maltese colour associated with the gene **d**. The patch occurred between the white and black areas as an extension of the black cheek spot. This animal was descended from a stock of black Dutch-marked mice obtained from an English fancier in 1927 and inbred in our laboratory for three generations. One great-grandparent of the mosaic was heterozygous for dilution, so the mosaic was mated with dilutes. These tests produced twenty-four intense young showing that he was homozygous **DD**. He proved to be heterozygous for brown (**Bb**), but this probably had no connection with the mosaic spot. When one year old the blue patch began to fade and became nearly white through the ingrowth of white and of much paler blue hairs. He was then tested for an albino allelomorph but produced only 4 $\mathbf{C^+}$ young by an albino mate. When crossed with his mother, sister and two daughters he produced twenty-two Dutch-marked offspring but no mosaics. In the absence of a complete analysis we have no evidence that a genetic change was involved, and the blue patch may be ascribed to some accident in development affecting the pigment-forming mechanism in the blue area. This case is not included in the survey of mammalian mosaics which follows.

[2] Bittner's (1932) case of a dilute brown mouse with intense brown back is not included since from the description given it showed no spots of contrasting colour and there is no conclusive evidence of genetic differences in the different areas.

character is unknown. Of the remaining eighteen cases occurring in spotted animals, fifteen involved no new mutation since they occurred in heterozygotes in which the recessive effect appeared in part of the coat (Wright (1926), three cases; Castle (1922), one case; Pincus (1929), three cases; Fisher (1930), one case; Castle (1929), seven cases in one family. The simplest assumption that these are all due to loss of the chromosome carrying the dominant allelomorph in a somatic mitosis is not disproved in any of these cases. In all except one of these cases (Castle's tricolour rat (1922)) the mutant area appears as a *single patch*, as would be expected if a single abnormal cell division involving non-division or non-disjunction of one chromosome had occurred in the direct ancestry of this tract. The three cases occurring in spotted animals to which this interpretation is not applicable include (1) Wright's two intense-dilute mosaic guinea-pigs which were homozygous for the colour genes involved but which Wright concluded might be due to the normal spotting effect of the tortoise-shell gene $\mathbf{e^p}$ in conjunction with other modifiers, and (2) Wright's homozygous agouti guinea-pig **AA**, which showed a patch of non-agouti **aa** involving either loss of both **A** genes or mutation to a dominant black. None of these cases is sufficiently clear to constitute an important exception to the general relationship indicated between abnormal somatic mitoses and white spotting. The suggestion which emerges is thus that somatic mitoses may more frequently be irregular in animals with white spotting.

In only one case has clear evidence of the transmission of an hereditary tendency to mosaicism been reported. This is Castle's (1929) case of a black and white spotted rabbit heterozygous for blue dilution (**Dd**) which showed a large patch of blue fur. The mosaic condition appeared also in three of his seventy-eight progeny which might have shown the condition. Similarly a mosaic son sired three mosaics out of eighty-three progeny which might have shown the condition. Castle has attempted to explain the transmission of mosaicism by assuming that a mosaic gene consisting chiefly of the **D** allelomorph, but with some of the **d** allelomorph, has arisen, presumably by some mutative process. This, of course, provides no explanation of the developmental basis of mosaicism, which must still be assumed to lie in some mechanism for segregating the two effects of the one gene into different cells, a process more difficult to conceive than that which segregates two allelomorphs to different cells, or which merely eliminates one of them from one daughter cell. A mosaic gene acting within one cell might more often be expected to produce an effect intermediate between the effects of the two allelomorphs of which

it is composed, as in the zygotic compounds of most allelomorphic series, rather than to become partitioned sharply in different cells, merely by reason of its mosaicism. It would seem more in accord with the facts to assume that what is transmitted in the above case is an hereditary condition favouring instability in somatic mitoses, such as the effect associated with the claret gene of *Drosophila simulans* (Sturtevant, 1929) or with the numerous minute mutations of *D. melanogaster*. The established processes of mutation and of chromosome behaviour seem adequate to explain the present data from mammalian colour mosaics.

REFERENCES.

Bittner, J. J. (1932). "A color mosaic in the mouse." *Journ. Heredity*, **23**, 421.

Castle, W. E. (1912). "On the origin of a pink-eyed guinea-pig with colored coat." *Science*, N.S. **35**, 508–10.

—— (1922). "On a non-transmissible tricolor variation in rats." *Carnegie Inst. Publ.* No. 320, pp. 51–4.

—— (1929). "A mosaic (intense-dilute) coat pattern in the rabbit." *Journ. Exp. Zool.* **52**, 471–80.

Fisher, R. A. (1930). "Note on a tricolour (mosaic) mouse." *Journ. Gen.* **23**, 77–81.

Gates, W. H. (1931). "Linkage of shaker, albinism and pink-eye in the housemouse." *Z. ind. Abst. u Vererb.* **59**, 220.

Pincus, G. (1929). "A mosaic (black-brown) coat pattern in the mouse." *Journ. Exp. Zool.* **52**, 439–41.

Sturtevant, A. H. (1929). "The claret mutant type of *Drosophila simulans*." Zeitschr. wiss. Zool. **135**, 323–56.

Wright, S. (1926). "Mutational mosaic coat patterns of the guinea-pig." *Genetics*, **11**, 333–51.

EXPLANATION OF PLATE XXIII.

Mouse with mosaic coat-colour.