

FURTHER EXPERIMENTS ON THE INHERITANCE OF COAT-COLOUR IN RABBITS.

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IN an earlier paper published in this Journal¹ I gave an account of some experiments with rabbits in which it was shewn that the agouti pattern might in certain cases result from the mating of black with black. Until then all the evidence from mice, rats and guinea pigs, as well as from rabbits, pointed to the relation between agouti and black being a simple Mendelian one in which agouti was dominant. The unexpected appearance of agoutis in litters from black parents led to the framing of a hypothesis which may be briefly recapitulated before giving an account of the further experiments which I have made during the past two years. On that hypothesis I suggested that we were dealing with three factors, viz.:

- A, the agouti factor which inhibits the full production of black pigment, turning black into agouti, and tortoise into yellow;
- E, a factor for the extension of the melanic pigment which turns yellow into agouti and tortoise into black; and
- D, a factor which inhibits the operation of the agouti factor, causing the animal to appear almost or quite black even if the agouti factor be present.

In order to explain the experimental results it was suggested that complete coupling occurs between the factors D and E, so that animals of the constitution $DdEe$ produce only the gametes DE and de. The

¹ *Journal of Genetics*, 1912, Vol. II. pp. 221—238.

factor **A** however behaves in segregation independently of **D** and **E**. Owing to this coupling no animal contains **D** unless it also contains **E**, and no animal which is heterozygous for **E** can be homozygous for **D**. In other words, if the coupling is complete, animals of the constitution **DDee**, **Ddee**, and **DDEe** should not be found.

The presence or absence of **D** makes no difference in the appearance of a rabbit which contains **E**, but lacks the agouti factor, **A**. Whether **D** is present or not such animals are normal blacks. But when **A** is present in animals homozygous for **E** their appearance differs according as they are homozygous or heterozygous for **D**. When heterozygous the animal is mainly black with a slight sprinkling of agouti hairs, which are most numerous at the back of the neck (cf. *Journ. Gen.* 1912, Pl. XII, fig. 2); when homozygous it must be supposed to be full black¹. Further, the appearance of an animal containing both **D** and **A** differs according as it is homozygous or heterozygous for **E**. If homozygous it is an agouti-black: if heterozygous it is a normal black in appearance.

Hence the zygotic constitutions of animals containing **D**, **E**, and **A** are limited to the following:

- | | | |
|------------------|---|---------------|
| 1. DDEEAA | } | normal black. |
| 2. DDEEAa | | |
| 3. DdEEAA | } | agouti-black. |
| 4. DdEEAa | | |
| 5. DdEeAA | } | normal black. |
| 6. DdEeAa | | |

At the time when the earlier results were given in 1912, all of the above six classes had been experimentally identified with the exception of the first. As the identification of these different classes is critical for the hypothesis adopted, experiments were begun in order to find the animal of the constitution **DDEEAA**. It was shewn that when agouti-blacks of the constitution **DdEEAa** were bred together they gave, as the hypothesis demanded, blacks, agouti-blacks, and agoutis closely in the ratio 7:6:3. Such animals produce the four types of gamete, **DEA**, **DEa**, **dEA**, **dEa**, and the results of two such gametic series meeting is shewn in the subjoined scheme:

¹ Critical evidence in favour of this view was lacking in 1912 but has since been obtained (p. 39).

DEA DEa Black	DEA DEa Black	DEA dEA Agouti-black	DEA dEa Agouti-black
DEa DEA Black	DEa DEa Black	DEa dEA Agouti-black	DEa dEa Black
dEA DEA Agouti-black	dEA DEa Agouti-black	dEA dEA Agouti	dEA dEa Agouti
dEa DEA Agouti-black	dEa DEa Black	dEa dEA Agouti	dEa dEa Black

Fig. 1.

As indicated in the scheme, the 7 blacks should be zygotically of five different sorts, viz.:

- 1 DDEEAA.
- 2 DDEEEa.
- 1 DDEEEa.
- 2 DdEEaa.
- 1 ddEEaa.

Crossed with an animal of the constitution *ddEEaa*, the last three classes should give only blacks; those of the constitution *DDEEAA* should give equal numbers of blacks and agouti-blacks; while those of the constitution *DDEEAA* should give agouti-blacks only. The three different classes as revealed by this test should be in the proportion 4:2:1. Moreover no agouti should make its appearance in any of these crosses.

Blacks resulting from the matings between agouti-blacks of the constitution *DdEEAa* were accordingly saved and tested by crossing with the chocolate buck whose history was given in the earlier account (*Journ. Gen.* 1912, p. 233). With respect to the factors in question he was of the constitution *ddEEaa*.

In all, 14 does were tested in this way and gave the results shewn in Table I.

The results tally closely with expectation which demands that the three classes should be found in the ratio 4:2:1. The actual numbers, 8:5:1, are not far removed from the expected 8:4:2. Moreover the case of *F* 238 shews that the animal of the constitution *DDEEAA* is

TABLE I.

Blacks ex agouti-black × agouti-black.

<i>F</i> 188 × choc.	gave	4	black	} DDaa Ddaa ddaa
<i>F</i> 189	„ „	2	„	
<i>F</i> 190	„ „	7	„	
<i>F</i> 194	„ „	6	„	
<i>F</i> 198	„ „	6	„	
<i>F</i> 199	„ „	4	„	
<i>F</i> 212	„ „	3	„	
<i>F</i> 213	„ „	5	„	
<i>F</i> 93	„ „	4	black + 5 agouti-black	} DDAA
<i>F</i> 94	„ „	4	„ +1 „	
<i>F</i> 207	„ „	4	„ +1 „	
<i>F</i> 215	„ „	1	„ +2 „	
<i>F</i> 217	„ „	4	„ +2 „	
<i>F</i> 238	„ „	10	agouti-black	DDAA

a normal black in appearance. This deduction, which could only be assumed in the earlier account (*Journ. Gen.* 1912, p. 230), is now confirmed by direct experiment.

It was previously pointed out that agouti-blacks must, on the hypothesis put forward, be always heterozygous for *D*. In addition to the eight *F*₂ rabbits already tested and shewn to fulfil this condition (*Journ. Gen.* 1912, p. 233), five *F*₃ agouti-blacks (*F* 84, *F* 85, *F* 94, *F* 104, and *F* 150) from agouti-black parents have also been tested. In each instance the animal was shewn to contain *A* and to be heterozygous for *D*.

These further results all served to confirm the view previously advanced, and the chief remaining point of interest was to determine whether the coupling between the factors *D* and *E* must be regarded as complete, or whether it is of the nature of a partial coupling of high intensity. For this purpose a number of rabbits of the constitution *DdEeAA* were bred (see *Journ. Gen.* 1912, p. 233) and mated with animals containing neither *D* nor *E*. The appearance of a few agoutis from such matings would indicate that the coupling was partial. The facts obtained are shewn in Tables II and III¹.

Some further data are also given from the matings of four does of the constitution *DdEeAa* (Table IV). If the coupling between

¹ A few of the data given in Tables II—IV have already been recorded (*Journ. Gen.* 1912, pp. 233, 234). The bulk of them however are new.

D and E is partial, agoutis are also to be expected here, though of course the chance of their appearing is only half as great as in the case of animals which are homozygous for A.

As these Tables shew, 476 rabbits in all have been bred from animals of the constitution DdEe (which also contain A) when mated

TABLE II.

		♂ E 31 (ddeeAa)		♂ F 235 (ddeeAA)		Orange ♂ (ddeeaa)		♂ 74 (ddeeaa)		♂ G 35 (ddeeAA)		Totals	
		blk	yell.	blk	yell.	blk	yell.	blk	yell.	blk	yell.	blk	yell.
		DdEeAA ♂ ♀	F 63	2	2	2	8	2	3	1	2	—	—
F 67	5		2	9	10	—	—	—	—	—	—	14	12
F 148	2		4	8	8	—	—	—	—	1	1	11	13
F 231	—		—	2	2	5	2	—	—	—	—	7	4
F 232	—		—	5	8	2	2	—	—	2	3	9	13
F 233	—		—	5	1	2	4	3	2	—	—	10	7
G 20	—		—	—	—	—	—	—	—	3	1	3	1
Totals											...	61	65

TABLE III.

	♀ ddee	♂ ♂ DdEeAA							
		♂ F 54		♂ F 20		♂ G 12		♂ G 21	
		blk	yell.	blk	yell.	blk	yell.	blk	yell.
F 128	"	—	—	3	2	5	10	—	—
F 222	"	—	—	—	—	—	—	6	1
F 223	"	—	—	3	0	3	7	8	9
F 230	"	—	—	2	4	—	—	—	—
F 236	"	—	—	—	—	2	1	2	6
G 17	"	—	—	—	—	6	5	7	9
G 18	"	—	—	0	2	6	8	6	4
G 19	"	—	—	3	0	1	2	1	1
G 22	"	—	—	—	—	4	3	6	6
G 23	"	—	—	—	—	1	1	9	8
G 30	"	—	—	—	—	8	10	—	—
G 41	"	—	—	—	—	—	—	4	5
G 42	"	—	—	—	—	3	2	1	5
G 43	"	—	—	—	—	8	8	—	—
G 49	"	—	—	1	0	—	—	—	—
Totals		7	3	14	11	47	57	50	54
Totals		...		} Black 118		} Yellow 125			

TABLE IV.

		♂ E 31 (ddeeAa)			♂ (ddeeaa)			♂ F 235 (ddeeAA)	
		blk	yell.	tort.	blk	yell.	tort.	blk	yell.
F 22	♀ DdEeAa	7	5	2	10	10	8	4	2
F 62	„	3	1	1	4	2	3	3	2
F 66	„	3	2	1	2	1	2	—	—
F 69	„	3	6	3	9	4	4	—	—
Totals ...		16	14	7	25	17	17	7	4

with animals of the constitution **ddee**. Since no agouti has appeared it must be inferred that **dEA** gametes (and also **Dea** gametes) were not formed. Hence it must be supposed that the coupling between **D** and **E** is complete, or that if partial, it is of very high intensity. At present we have no grounds for supposing that it is not complete.

The Chocolate Series.

In my earlier paper I gave a brief account of some experiments with a chocolate rabbit, for which I was indebted to the kindness of Dr Hagedoorn (*loc. cit.*, p. 235). Further work has confirmed the view that chocolate in the rabbit, as in the mouse, behaves as a simple recessive to black, and that the black series of colours is paralleled by a corresponding recessive chocolate series. Chocolate × yellow gives agoutis in F_1 , and these agoutis bred together give in F_2 agouti, black, yellow and tortoise, and also cinnamon, chocolate, dilute cinnamon, and orange—the corresponding colours in the chocolate series. The considerable mass of data accumulated during the past few years amply confirms the view that the chocolate series runs strictly parallel with the black series, differing from the latter simply in lacking the factor for black (**B**).

The relations of the chocolate series to the factor **D** are however of interest, and may be considered in more detail. A pedigree is given in Fig. 2 in order that the somewhat complicated experiments may be more easily followed. An F_2 agouti-black doe of the constitution **DdEEAa** was mated with the chocolate buck received from Dr Hagedoorn. Since her gametic output is **DEA**, **DEa**, **dEA**, **dEa**, she gave, as was expected, blacks, agoutis, and agouti-blacks, all of which were of course heterozygous for **B**. The blacks and agoutis

Moreover the proportion in which these 6 forms occurred is that expected on hypothesis. The agouti-black ♀♀ of the constitution **DdEEAaBb** must be supposed to produce 8 kinds of gamete—**DEAB**, **DEAb**, **DEaB**, **DEab**, **dEAB**, **dEAb**, **dEaB**, **dEab**—while all of the gametes of the chocolate ♂ are of the type **dEab**. The result of such a cross is shewn in Fig. 3, and, as Table V indicates, the expectation of the 6 classes in the ratio 1 agouti, 1 agouti-black, 2 black, 1 cinnamon, 1 deep cinnamon, 2 chocolate is closely realised in fact.

DEAB dEab Agouti-black	DEAb dEab Dark Cinnamon	DEaB dEab Black	DEab dEab Chocolate
dEAB dEab Agouti	dEAb dEab Cinnamon	dEaB dEab Black	dEab dEab Chocolate

Fig. 3.

It is evident from Fig. 3 that the chocolates (as also the blacks) produced should be of 2 sorts, viz. those heterozygous for **D**, and those which do not contain this factor. In order to put this to the test two of these chocolates were mated with a homozygous agouti ♂ (*F* 141)¹. One of these chocolates (*E* 239) gave 9 agouti young and was therefore of the constitution **ddEEaabb**. The other (*G* 51) produced 2 agoutis and 2 agouti-blacks. Her constitution must therefore have been **DdEEaabb**. The experiment establishes the fact that an animal which is pure chocolate in appearance can carry the factor **D**. All the evidence shews that there exists a chocolate series containing the factor **D** which is strictly parallel to the corresponding black series.

Here mention may be made of a few experiments which I carried out with blue rabbits, from which it is clear that a blue rabbit may carry the factor **D** in the same manner as a black. Thus a blue doe (*F* 211) was made which, when crossed with the original chocolate buck (cf. p. 42), gave 2 blacks, 1 agouti-black, and 1 agouti. Evidently the absence of the factor which turns blue into black does not affect the relations of the factor **D**.

¹ With 2 chocolate ♀♀ this buck gave 16 agouti young, with a chocolate Himalayan he gave 6 agoutis, and with an orange (=dilute chocolate) doe he gave 9 agoutis. No offspring of any other colour were produced and there can therefore be no doubt that in constitution he was **ddEEAABB**.

Discussion.

From the preceding account it is clear that all the facts observed in this series of experiments can be interpreted on the "Presence and Absence" hypothesis if it be supposed that complete coupling obtains between the factors D and E. There is however another hypothesis—that of "Multiple Allelomorphs"—which has been put forward in connection with this and similar cases¹. The difference between the two explanations may best be brought out by considering the instance used by Sturtevant (10) in a recent discussion of the problem. It was pointed out some years ago by Hurst and by Castle that albinism behaves as a simple recessive to self-colour. Both of these observers have further shewn that the Himalayan pattern also behaves as a simple recessive to self-colour. Castle's experiments again proved that the Himalayan pattern is a simple dominant to albinism. Nevertheless Himalayans do not appear in F_2 families from self-colour \times albino. Sturtevant has pointed out that the genetic behaviour of these colour varieties is covered by either of two hypotheses.

- A. On the "Presence and Absence" hypothesis we must suppose that two factors are concerned, viz. **C**, a colour producer which is present in the self-coloured and in the Himalayan rabbit, and **S**, a factor which turns the Himalayan into self-colour. Zygotically therefore the three forms may be represented thus:

Self-coloured	...	CCSS ,
Himalayan	...	CCss ,
Albino	ccss .

The albino must be supposed to lack **S** because no case is known of a self-coloured rabbit being produced from the mating Himalayan \times albino.

In order to account for the non-appearance of Himalayans in F_2 from self-colour \times albino, we must suppose that there is complete coupling between **C** and **S**, so that F_1 rabbits of the constitution **CcSs** produce only gametes of the form **CS** and **cs**.

¹ The hypothesis would appear to have been put forward independently by Morgan (7) and by Wilson (12) in 1913 (see also note on p. 46).

- B. On the "Multiple Allelomorph" hypothesis it must be supposed that we are dealing with three definite factors, any one of which is allelomorphic to any other, so that a given zygote cannot contain more than two of the three. If, following Sturtevant, we denote these factors as S, H, and A, the zygotic constitution of the three varieties becomes:

Self-coloured	...	SS,
Himalayan	HH,
Albino	AA.

The heterozygous self-colour which throws Himalayans is SH, and that throwing albinos is SA: while HA represents the constitution of a Himalayan which throws albinos¹.

It is clear that either hypothesis will cover all the facts. It is equally clear that the "Multiple Allelomorph" hypothesis carries with it the abandonment of the "Presence and Absence" hypothesis, not only in such cases as the present, but in all cases where Mendelian inheritance has been shewn to hold good. For there would appear to be no reconciliation possible between a hypothesis which interprets characters behaving as alternate in heredity, in terms of factors which are alternative in the gamete, and one in which an alternate pair of characters is interpreted in terms of the presence or absence of a single factor. In attempting to decide which of the two views is to be preferred, the rabbit case dealt with in the earlier part of this paper is not without interest.

In Sturtevant's discussion of the matter he points out that in these cases, where a given character appears to act as a simple dominant to two different recessives, and where one of these recessives also behaves as a simple dominant to the other, the three characters concerned are closely related physiologically. From this he argues that a given factor is a complex thing which may undergo modifications affecting its manifestation in the zygote without at the same time affecting its allelomorphic distribution in gametogenesis. In the rabbit case for example the factor for self-colour, which we may denote by X, is normally allelomorphic to itself. As the result of some unknown change in the constitution of the factor it loses the property of

¹ Wilson (1913) has also advocated the hypothesis of Multiple Allelomorphs in connection with the Agouti-black rabbit. On his interpretation the factor X (= DE) always turns a rabbit into a black or an agouti-black. This however is not in accordance with fact, since the factor in question can be carried by a chocolate (cf. p. 44).

bringing about complete pigmentation, passing into the condition X' , so that animals which are homozygous for the factor after the change has occurred ($X'X'$) display the Himalayan pattern in place of self-colour. Again, as the result of some other unknown change it becomes X'' and loses the property of bringing about any pigmentation, so that animals homozygous for the factor in this condition ($X''X''$) are albinos. Nevertheless both X' and X'' remain equivalent to X and to one another in the mechanism of factorial distribution, i.e. any one of the three is allelomorphic to any other one¹.

Of the cases cited by Sturtevant in support of his contention three, viz. the Himalayan rabbit case, the case of the green, variegated, and *chlorina* foliage in *Aquilegia*, and that of the red, eosin, and white eye in *Drosophila*, are certainly favourable to it. In all of them there is a series of three forms of diminishing pigmentation, and the genetic relations are similar in all².

In this connection the rabbit case dealt with in the earlier part of this paper is of interest, as it differs in some respects from the group of cases just mentioned. On the presence and absence hypothesis the two factors D and E are, so far as is known, completely coupled, and the three gametic possibilities are therefore DE , dE , and de . To bring this case into line with those discussed above we should have to refer these three possibilities respectively to the three allelomorphs X , X' , and X'' . The subjoined table represents the zygotic formulae of the various possible combinations on these two hypotheses, both when the agouti factor is present and when it is absent.

Without the agouti factor		With the agouti factor	
DDEE	black XX	DDEEAA	black XXAA
ddEE	black $X'X'$	ddEEAA	agouti $X'X''AA$
ddee	tortoise $X''X''$	ddeeAA	yellow $X''X''AA$
DdEE	black XX'	DdEEAA	agouti-black $XX'AA$
DdEe	black XX''	DdEeAA	black $XX''AA$
ddEe	black $X'X''$	ddEeAA	agouti $X'X''AA$

¹ Sturtevant does not suggest what the nature of these changes in the factor may be—whether some internal change involving merely a rearrangement of its constituents and analogous to isomerism in chemistry, or the definite loss of some constituent in each case. The latter view would practically mean the application of the presence and absence hypothesis to certain of the constituents of the factor itself.

² The case of Emerson's beans also cited by Sturtevant may perhaps be of a similar nature, but until the relation of the green-leaved-yellow-podded to the yellow-leaved-yellow-podded form has been investigated this cannot be regarded as certain.

When the agouti factor is present the three homozygous forms XX , $X'X'$, $X''X''$ form what may be regarded as a series of diminishing intensity for the melanic pigment, viz. black, agouti, yellow. In the absence of the agouti factor the series is black, black, tortoise. Though the first term is now in appearance indistinguishable from the middle one, it is theoretically possible to regard it as potentially of a more intense black, the difference between the two being rendered visible when A is inserted as an indicator. So far the manifestation of pigment is not at variance with the view that the three allelomorphs X , X' and X'' form a series of diminishing intensity. A difficulty however arises when we come to consider certain of the heterozygous forms. $XX'AA$ is agouti-black, and since X' must be regarded as a factor for higher pigment intensity than X'' we should expect $XX''AA$ to shew less intensity of melanic pigment than $XX'AA$. It should be something lighter than agouti-black, whereas it is actually full black. We cannot therefore regard the factors X , X' , and X'' in this case as forming a series of diminishing intensity for melanic pigmentation, and Sturtevant's argument, applicable in the cases of the Himalayan rabbit, the eye-colour of *Drosophila*, and the variegated *Aquilegia*, here falls to the ground.

Apart from the difficulty of interpreting the experiments dealing with the D factor in rabbits in terms of the "Multiple Allelomorph" hypothesis, there seem to me to be other reasons for treating all of these cases as examples of complete coupling and retaining the interpretation in terms of the "Presence and Absence" hypothesis. In this connection some recent work of Tanaka on silkworms is much to the point. In his account of the inheritance of the two larval characters striping (S) and yellow (Y) as opposed to non-striping (s) and white (y), Tanaka shews that the experimental data can only be interpreted on the supposition that there is partial coupling in the male gametes and complete coupling in the female gametes. The male gametic series is of the form $3SY : 1Sy : 1sY : 3sy$ (or $2SY : 1Sy : 1sY : 2sy$), while the female series is of the form $SY : sy$. Unless we are to suppose that the hereditary mechanism is of a different order in the two sexes, we can scarcely avoid the conclusion that the complete coupling exhibited in the female is but a particular form of the partial coupling found in the male series of gametes. And if we accept the hypothesis of complete coupling in this case it seems not unreasonable to extend it to other cases where partial coupling for the factors concerned is hitherto unknown.

Nor should it be forgotten that more ample data may shew these debatable cases to be examples of partial coupling of very high intensity. The appearance of a single Himalayan in F_2 from the cross of self-colour \times albino of an eosin-eyed *Drosophila* ex red eye \times white would, as Sturtevant admits, invalidate the "Multiple Allelomorph" hypothesis. Cases are now known where the coupling between two factors, though not quite complete, is of very high intensity. In sweet peas for example the coupling between the factor for blue (B) and that for the erect standard (E) is such that in plants heterozygous for both factors, the gametic series must be supposed to be 127BE : 1Be : 1bE : 127be. Such plants crossed with be pollen would give but a single specimen of each of the two rarer classes (blue hood and red erect) in 256 plants. If the coupling were of even higher intensity it might easily be regarded as complete unless very large numbers were raised. As time goes on the phenomenon of partial coupling and repulsion is turning out to be a widely spread one among animals as well as plants. Whatever the explanation may turn out to be ultimately, it seems reasonable to regard cases of apparent complete coupling as falling into the general scheme until we have definite evidence to the contrary. It would appear premature therefore to reject the "Presence and Absence" hypothesis for that of "Multiple Allelomorphs" until we are in possession of facts which compel us to take this step. We certainly do not possess such facts at present.

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