

ON THE SERIES OF ALLELOMORPHS CONNECTED WITH THE PRODUCTION OF BLACK PIGMENT IN RABBITS.

By R. C. PUNNETT, F.R.S.

INTRODUCTION.

SOME years ago (1912) I showed that there existed a black form of the rabbit which behaved as dominant to agouti, whereas the normal black in this species is a recessive. I suggested that this was due to a dominant factor, **D**, which inhibited the action of the agouti factor, **A**, and that this factor was completely (or almost completely) linked with the factor for extended pigmentation (**E**) such as occurs in normal blacks and agoutis, as opposed to that for restricted pigmentation (**e**) found in tortoises and yellows.

With the advent of the conception of multiple allelomorphs Wilson (1913) suggested that the simplest way of regarding this case was to suppose that dominant black, normal black, and tortoise depended upon three allelomorphs, and that in the presence of the agouti factor (**A**) the series became black, agouti, and yellow.

More recently independent experiments by Castle and by myself (1924) showed that the factor (**J**) for the mixed yellow and black coat pattern of the Japanese rabbit must be regarded as allelomorphic to black (**E**) and tortoise (**e**). In the present paper evidence is brought forward in favour of the factor for dominant black (**D**) also being allelomorphic to Japanese, as indeed was to be expected.

Some years ago Onslow (1922) worked out the genetics of the "steel" rabbit, a dark agouti form with the belly pigmented instead of white as in the ordinary agouti. He showed that steel is a heterozygous form, and that, in material homozygous for **A**, steel \times steel gave blacks, steels and agoutis in the ratio 1 : 2 : 1. As Onslow pointed out, the simplest explanation is to suppose that here again we are concerned with a factor for dominant black (**D'**), allelomorphic to that for normal recessive black (**E**). In the presence of **A** the **D'D'** rabbit is black, the **D'E** is steel, and the **EE** rabbit is agouti. At present we may regard the "steel" factor (**D'**) as distinct from the dominant black factor (**D**), for reasons which will appear later.

Hitherto **D'** has not been related to any member of the multiple allelomorphic series **D**, **E**, **J**, **e**, except to **E**. The object of the following

experiments was to test the relations of **J** to **D** and **D'**, in the light of Castle's work and my own with the Japanese rabbit.

MATERIAL.

I had long since ceased to breed dominant blacks, but I was fortunate in being able to procure from my friend, Mr T. H. Riches, a buck descended from my original material. He turned out to be **DDaa**, and also heterozygous for blue and for the Himalayan pattern.

For my "steel" material I am indebted to the Hon. Mrs Onslow, who kindly sent me a pair when her husband's rabbits were dispersed after his death. Unfortunately they proved to be sterile with one another, and very shy breeders with other stock. The **D'** material used was extracted from crosses derived from the original steel buck.

For the work I had in mind I should have liked to use **DDAA** and **D'D'AA** animals, but to have built up strains of the above constitutions in a reasonable time was not possible with the limited accommodation available. For this reason the experiments are more numerous and less orderly than I could have wished. To set them all out would be tedious and I shall, therefore, give selected evidence only. But it should be stated that the various miscellaneous data accumulated are in accordance with the interpretation given in terms of a series of multiple allelomorphs.

THE RELATION OF **D** TO **J**.

The buck obtained from Mr Riches (σ 144) was mated with a Japanese doe (♀ 138) of the constitution **AaJJ**, and gave eight blacks. Of these four ♀♀ and one σ were used to produce the F_2 generation set out in Table I.

TABLE I.

	Black	Blue	Choco- late	Lilac	Himalay- ans	Japa- nese
♀ 170 \times σ 169	10	—	—	—	—	7
♀ 171 \times "	6	—	4	1	4	2
♀ 172 \times "	6	1	1	1	5	2
♀ 173 \times "	3	—	2	—	1	2
	25	1	7	2	10	13
	35					

The appearance of blues and Himalayans is due to σ 144, which was known from other evidence to have carried these characters, while the chocolate came into the cross through ♀ 138. The Himalayans were killed early before it could be decided whether they corresponded to the

self-coloured or to the Japanese type. Of the rest there were thirty-five self-coloured and thirteen Japanese, a fair approximation to the 3 : 1 ratio expected on the assumption that **D** and **J** are allelomorphic. Further, although some of the F_1 animals probably carried **A**, no agouti-marked animal appeared in F_2 . This also is in accordance with expectation on the assumptions that **D** and **J** are allelomorphic, and that the **DJA** animal shows no agouti ticking. Evidence for the latter assumption will be given below (cf. p. 272).

Twelve of the F_2 blacks were then crossed with Japanese, nearly all of which were homozygous for black (**BB**). The results of these matings are set out in Table II.

TABLE II.

F_2 black	Japa- nese		Black	Choco- late	Japa- nese
♂ 211 × ♀ 188		gave	2	—	2
♂ 212 × {♀ 166 ♀ B 11}		"	8	—	—
♀ 228 × ♂ 227		"	4	—	—
♂ 229 × {♀ 215 ♀ B 12}		"	5	—	10
♀ 234 × ♂ 163		"	2	—	3
♀ 235 × ♂ 165		"	2	—	5
♀ 236 × ♂ 187		"	6	—	1
♀ 237 × ♂ 187		"	10	—	—
♀ 238 × ♂ 227		"	5	—	—
♀ B 2 × {♂ 227 ♂ 187}		"	10	—	—
♂ B 3 × ♀ 217		"	1	1	1

Of the twelve animals tested four, viz. ♂ 212, ♀ 237, ♀ B 1 and ♀ B 2 were evidently homozygous for **D**; six, viz. ♂ 211, ♂ 229, ♀ 234, ♀ 235, ♀ 236 and ♂ B 3 were heterozygous; while ♀ 228 and ♀ 238 were not sufficiently tested for certainty. The six definite heterozygotes gave in all nineteen self-coloured and twenty-two Japanese, a near approach to the equality expected on the assumption that **D** and **J** are allelomorphic.

Of the Japanese rabbits used one, ♂ 187, was known from other evidence to have been heterozygous for **A**. With ♀♀ 236, 237, and B 1 he gave twenty-nine self-blacks, none of which had any trace of agouti ticking. Since about 50 per cent. of these animals must have carried **A**, it is clear that the **DJA** rabbit is a full black in appearance. In this respect the factor for Japanese (**J**) behaves similarly to that for tortoise (**e**) (cf. Punnett, 1912).

These experiments are consistent with the view that **D** and **J** are allelomorphic.

THE RELATION OF **D'** TO **J**.

As already stated the **D'** factor was derived from one of Onslow's steels, viz. ♂ 137. Mated with a yellow doe (♀ 119) he gave agouti-blacks¹ and agoutis. Of these an agouti-black doe (♀ 178) and an agouti buck (♂ 176) were bred together and gave in all:

2 agouti, 1 black, 3 steel, 1 agouti-black
 1 cinnamon, 1 chocolate, 1 chocolate steel, 1 dark chocolate agouti } 5 yellow or orange,

where expectation, on the assumption that **D'**, **E** and **e** are allelomorphic, would be

2.25 agouti, 2.25 black, 2.25 steel, 2.25 agouti-black
 0.75 cinnamon, 0.75 chocolate, 0.75 steel, 0.75 dark chocolate agouti } 4 yellow or orange.

It is remarkable that, even in the small number bred, all of the expected classes appeared, and closely in the proportion expected. As shown in Pedigree I (p. 269), two of the steels, viz. ♂ 219 and ♀ 221, were subsequently used for breeding purposes, and a certain number of the animals bred were analysed by means of various matings. The results throughout were found to be consistent with the view that **D'**, **E**, **J** and **e** behave as multiple allelomorphs.

One further experiment may be referred to in more detail. From Pedigree I it will be seen that ♂ 219 was mated with a Japanese doe (♀ 188) with whom he gave three agoutis, three agouti-blacks and two blacks. One of the agouti-blacks, ♀ B 34, was tested and found to be **AABBD'J** in constitution. She was then mated with ♂ B 48, a black of the constitution **aaBBEE**. With him she gave eleven agoutis and eight steels. On the assumption that **D'**, **E** and **J** are allelomorphic these agoutis should all be **AaBBEJ**, and the steels all **AaBBD'E** in constitution. The agoutis bred together should give agoutis, blacks and Japanese in the ratio 9 : 3 : 4. Actually two does (♀ B 108 and ♀ B 110) mated to the same buck (♂ B 112) gave seven agouti, three blacks and two Japanese. Again, the steels bred together should give agoutis, blacks, and steels in the ratio 3 : 7 : 6. Actually two does (♀ B 109 and ♀ B 111) mated to the same buck (♂ B 114) gave five agoutis, nine blacks, and six steels. In either case the results, both qualitative and quantitative, are in full agreement with the assumption that **D'**, **E**, and **J** are members of the same allelomorphic series.

¹ *I.e.* blacks with a small but variable amount of agouti ticking. The variety is figured in Vol. II, Pl. XII, fig. 2, of this *Journal*.

THE RELATION OF **D** TO **D'**.

To test the relation of **D** to **D'**, the steel buck (♂ 219) mentioned above (p. 268) was mated with two extracted dominant black does (♀ B 1 and ♀ B 2) both of the constitution **aaBbDD** (cf. p. 267). Since ♂ 219 was genetically **AaBbD'E**, the expectation from this type of mating is as follows:

ABD'	ABE	AbD'	AbE	aBD'	aBE	abD'	abE
aBD	aBD	aBD	aBD	aBD	aBD	aBD	aBD
Black	Agouti-black	Black	Steel	Black	Black	Black	Black
ABD'	ABE	AbD'	AbE	aBD'	aBE	abD'	abE
abD	abD	abD	abD	abD	abD	abD	abD
Black	Steel	Chocolate	Chocolate Steel	Black	Black	Chocolate	Chocolate

This expectation is based upon the known facts (1) that the **DE** animal carrying **A** is agouti-black, (2) that the corresponding chocolate class is chocolate steel, and (3) that the **DE** animal containing **A** and also heterozygous for chocolate (**Bb**) is steel in appearance (cf. Punnett, 1912). It is also based on the assumption that the **DD'** animal carrying **A** is, like the **DD** animal, full black. Evidence in support of this assumption is given later (p. 271).

From this cross then five colour classes are expected, though two of them, the agouti-black and the chocolate steel, are rare, occurring only once in sixteen times. Actually four of the classes appeared among the twenty animals bred, the rare agouti-black class alone being unrepresented. On the other hand there were two of the corresponding chocolate class. The actual and expected figures are as follows:

	Black	Steel	Chocolate	Agouti-black	Chocolate steel
Actual	10	4	4	—	2
Expected	11.25	2.5	3.75	1.25	1.25

The correspondence between the expected and the actual result is sufficiently close in view of the small numbers bred.

A point of interest is that such steel rabbits as occur from this mating should be genetically **AaBbDE** in spite of the fact that the "steel" factor **D'** enters into the mating. The chocolate steels should also be **DE** in constitution. One of the steels (♀ B 16) and one of the chocolate steels (♀ B 39) were tested and were both found to be **DE** as expected.

Of the blacks three were tested and constitutionally determined as follows:

♀ B 17 was **AaB.DD'** (she might have been **BB** or **Bb**),
 ♀ B 18 „ **aaBBDE**,
 ♂ B 19 „ **AaBBDD'**.

The analysis of ♀ B 17 and ♂ B 19 proved the correctness of the assumption that the **DD'A** animal is a full black (cf. p. 270).

♂ B 19 was also mated with a black doe (♀ B 68) of the constitution **aaBBEE**. He produced two blacks, three agouti-blacks and one steel, these three classes only being expected in the ratio 2 : 1 : 1.

The above facts are all consistent with the view that **D** and **D'** segregate from one another, and are both members of the series of allelomorphs in which the five terms **D**, **D'**, **E**, **J**, and **e** have been hitherto identified.

GENERAL REMARKS.

A point of some interest in connection with this series of allelomorphs is the phenotypical expression of some of the heterozygous forms when **A** is present as an indicator. The earlier experiments on the **D** black showed that the **DD** form was always full black, the **DE** form agouti-black, and the **De** form again full black. This is true for **BB** animals. When heterozygous for chocolate (**Bb**) the **DE** form is dark steel, whereas the other two remain full black. These later experiments have confirmed the earlier results and have provided the additional information that the full black of the **DD'** and the **DJ** combinations is not affected by the presence of **A**. Koller (1930) recently showed that the formation of **E** black can be inhibited by **A** *in vitro*, whereas that of **D** black is not inhibited. Since the **DEA** rabbit is agouti-black we must suppose that **A** can produce a slight inhibitory effect on this combination. The **DJA** combination has always proved to be full black, nor is this surprising in view of the fact that the Japanese rabbit carrying **A** has never been found to show any trace of agouti marking (Punnett, 1924). **J** black, like **D** black, is what we may term a "refractory" black. Now since the **DeA** combination has always been found to be full black it would seem natural to regard the black pigment of the tortoise rabbit (**e**) as being also refractory in nature, and hence qualitatively different to **E** black to which it behaves as a simple recessive. But this view is negatived by the fact that the **eeA** rabbit is a yellow, with definite agouti ticking in spite of the paucity of black pigment, and with a white belly. We are driven to suppose that the **e** black, like the **E** black, is non-refractory. Why then should the **DEA** form, which contains two "full" colour factors, show some agouti ticking, while the **DeA** form, containing a "full" and a "dilute" one, is completely black?

Although what follows is admittedly crude speculation it is possible to look at the matter in the following way. The **DD** rabbit, with completely refractory black pigment, is always full black when **A** is present; and if it were possible to get a **DO** animal we should expect this also to be full black. The **DE** animal possesses the means of producing both refractory and non-refractory pigment, and the production of a certain amount of the latter enables **A** to bring about the formation of an agouti-black. When the **DE** animal is heterozygous for chocolate (**b**) the ticking is accentuated because chocolate is more readily inhibited than black¹. The **De** animal also possesses the means of producing both refractory and non-refractory pigment, but the power of producing the latter is here very much less than in the case of the **DE** animal. The power of producing refractory pigment, however, is presumably just as great, and it seems not unlikely that the full black of the **DeA** animal may be due to the almost exclusive formation of the refractory **D** pigment, which is possible in competition with **e** but not in competition with **E**.

We may now turn to similar considerations in connection with **D'**. Generally speaking **D'** may be regarded as a refractory black, but less refractory than **D**. The **D'D'A** animal is generally full black, but sometimes it shows traces of agouti marking even when homozygous for **B**². The **D'EA** animal is the "steel" of the fancy, and corresponds with the agouti-black in the **D** series. I have unfortunately no evidence as to whether the agouti markings are more pronounced when the animal carries chocolate. The **D'eA** rabbit in my experience is generally agouti-black, and indistinguishable in appearance from the **DEA** rabbit³. In some cases, however, rabbits which must have been of this constitution were recorded as full black before killing at a few weeks old. The ticking often does not develop until later, and it is possible that these rabbits might have become agouti-black. Whether **D'eA** rabbits can be full black when adult is at present undecided.

The **D'JA** animal may be either full black or agouti-black, and the

¹ This statement rests upon the observed facts (1) that the cinnamon (= chocolate agouti) is relatively less pigmented than the corresponding black agouti, and (2) that the chocolate steel, **AAbbDE**, is more obviously agouti marked than is agouti-black, **AABBDE**, its corresponding term in the black series.

² This was the case in ♂ B 21, cf. p. 269.

³ In 1918, when in the Isle of Wight, I came across a litter which contained agouti-blacks, agoutis, and yellows. At that time the **DEA** rabbit was the only form of agouti-black with which I was acquainted. I purchased one of the agouti-black does and mated her to a tortoise ♂. She gave agouti-blacks, agoutis, yellows and tortoisés, and must have been **AaD'e** genetically.

amount of ticking may vary from a trace to that characteristic of the normal agouti-black. I have hitherto been unable to relate these differences with any feature in the genetical constitution. They do not depend upon the animal being heterozygous for chocolate, since ♂ B 21 (**AABBD'D'**) with a yellow doe (♀ 220) gave seven young, all agouti-blacks. Since ♀ 220 was heterozygous for chocolate presumably some of the young were **BB** and others **Bb**. With a Japanese doe (♀ B 8) ♂ B 21 produced eleven young, also all agouti-blacks, *i.e.* the **D'JA** and the **D'eA** young from this buck were all similar in appearance.

Apart, therefore, from a certain amount of minor variation the reactions of **D'** in its combinations with **E**, **J**, and **e** are along similar lines to those of **D**, and such as might be expected if the chief difference between them lies in the fact that one is more refractory to the inhibitory activity of **A** than is the other.

Lastly, there is one further point which calls for brief mention, although its bearing is by no means clear. The ordinary agouti rabbit which carries **J**, *viz.* the **EJA** rabbit, frequently shows smudges of black which may occur on almost any part, including the light belly (*cf.* Punnett, 1924). On such heterozygotes the refractory Japanese black appears to be distributed independently of the **E** black, as indeed we know it to be in the **eJA** type. But although I have examined some dozens of agouti-blacks of the **D'JA** kind I have never been able to detect any irregularity in the distribution of the agouti ticking. In other words the **J** black does not give the appearance of being superimposed on the **D'** black as on the **E** and **e** blacks. It is tempting to suppose that **D'** is chemically more akin to **J** than are **E** and **e**, so that the combination **D'J** gives rise to a fairly uniform pigment; whereas, in the combinations **EJ** and **eJ**, **J** always acts independently of **E** and **e**. In any case this series of allelomorphs is one of the most interesting hitherto met with, and it is hoped that further light may eventually be thrown upon it by other experiments now in progress.

SUMMARY.

Earlier work had shown that of the factors connected with the production of melanic pigment in the rabbit two groups of three each, *viz.* **D**, **E**, **e**, and **J**, **E**, **e**, behaved as though in either case the three factors were allelomorphs. Evidence is now adduced to show that **D** and **J** are allelomorphs, and further evidence is brought forward to show that **D'**, the "steel" factor, also belongs to the same series of allelomorphs. The five members of the series differ in their reactions towards the inhibitory

agouti factor (**A**), **D** and **J** being completely refractory, **E** and **e** being non-refractory, and **D'** partially so. Various points are discussed in connection with the phenotypical manifestation of various combinations of the five allelomorphs.

I wish to acknowledge my indebtedness to the Government Grant Committee of the Royal Society, without whose assistance these experiments could not have been undertaken.

REFERENCES.

- CASTLE, W. E. (1924). "Genetics of the Japanese rabbit." *Journ. Gen.* XIV, 225-9.
 — (1925). "Heredity in rabbits and guinea-pigs." *Bibl. Gen.* I, 419-56.
 KOLLER, P. (1930). "On pigment formation in the D-black rabbit." *Journ. Gen.* XXII, 103-7.
 ONSLOW, H. (1922). "A note on the inheritance of the 'steel' coat-colour in rabbits." *Ibid.* XII, 91-9.
 PUNNETT, R. C. (1912). "Inheritance of coat-colour in rabbits." *Ibid.* II, 221-38.
 — (1915). "Further experiments on the inheritance of coat-colour in rabbits." *Ibid.* V, 37-50.
 — (1924). "On the 'Japanese' rabbit." *Ibid.* XIV, 231-40.
 WILSON, J. (1913). "Inter-alternative as opposed to coupled Mendelian factors: a solution of the agouti-black colour in rabbits." *Sc. Proc. Roy. Dubl. Soc.* XIII, 589-99.