A NOTE ON THE INHERITANCE OF THE "STEEL" COAT-COLOUR IN RABBITS.

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The work of Punnett, Hurst, Castle and others has treated of many colour varieties in the domestic rabbit, but the inheritance of the peculiar ticking of the hairs which produces the colour known as "steel" has apparently not yet been fully investigated. There appear to be more kinds of "steel" than one almost the same in appearance, though no doubt genetically quite different. Such, for example, are Punnett's "agouti-black," modified to steel by being heterozygous for *B*, the factor which converts chocolate into black. Again, there are the "*eisengrau*" of Pap, mentioned later, and no doubt more than one type of steel among Flemish and other breeds. The relationship of the various forms of "steel" to each other will no doubt be a problem of some difficulty, but it is one that calls for immediate solution.

The following communication deals with certain experiments which have been in progress for a considerable time, but owing to the many difficulties caused by the war, the results have not hitherto been published. The original animals were of the variety known as "steel Dutch," i.e. the coloured portions of the Dutch pattern were steel and the remaining portions white. The chocolate, black and yellow barring on a steel hair is not unlike that on the hairs of the common wild rabbit, except that steel hairs contain more black pigment, especially in the distal portions. These steel Dutch when crossed with some black English rabbits, produced, in addition to agouti, black self and English, a certain number of rabbits, in which the entire coat, including the belly and scut, had the same steel colour as the pigmented portions of the original Dutch parents. The rabbits were in fact steel selfs, and they supplied the material used in the following experiments.

A pair of these steels was first tested by crossing them with blues, to see whether they carried the dilution factor, which it was feared would complicate the results. When a number of steels had been obtained, which did not carry the dilution factor, they were mated

together, to see if it were possible to produce a rabbit homozygous for steel. On the supposition that steel is produced by the interaction of two factors *A* and *B*, steel, agouti and black would be produced in the ratio of 9 : 3 : 4. The steels would be of four types; 4 would produce all three forms, 2 would give steel and agouti, 2 would give steel and black, and 1 would give all steel. It was, however, found quite impossible to produce a homozygous steel, and although 28 rabbits of both sexes were tested, they all gave black and agouti as well as steel. In consequence of this it seemed probable that steel was itself a heterozygous form. A number of litters were accordingly obtained from matings of steel × steel, the results of which were as follows:

TABLE I.

Steel × *Steel.*

Mating ♀ × ♂	Steel	Agouti	Black
11 × 24 *a*	3	3	1
11 × 24 *a*	1	3	1
11 × 70	3	—	4
25 × 24 *a*	4	1	1
25 × 26	1	1	1
25 × 70	2	1	6
29 *a* × 24 *a*	3	1	3
32 *b* × 24 *a*	4	1	1
32 *b* × 24 *a*	3	3	—
32 *b* × 49 *b*	2	1	1
32 *b* × 49 *b*	3	1	1
38 *a* × 85 *a*	1	3	—
44 *b* × 24 *a*	3	2	—
44 *b* × 49 *b*	2	4	1
50 *a* × 28 *b*	2	2	1
50 *a* × 49 *b*	1	1	1
52 *c* × 28 *b*	4	1	3
54 *a* × 24 *a*	2	—	2
54 *a* × 49 *b*	2	—	—
54 *a* × 49 *b*	2	1	—
55 *b* × 24 *a*	3	—	1
56 *a* × 24 *a*	2	—	3
56 *a* × 24 *a*	4	2	1
56 *a* × 49 *b*	6	—	—
56 *a* × 49 *b*	—	—	4
60 *a* × 28 *b*	2	4	—
60 *a* × 28 *b*	1	1	2
70 *a* × 85 *a*	3	—	1
70 *a* × 85 *a*	1	—	1
79 × 28 *b*	3	—	—
80 × 28 *b*	1	—	—
80 × 24 *a*	4	2	—
84 × 57 *b*	4	1	—
87 *b* × 86 *a*	2	2	1
110 × 49 *b*	2	1	1
Totals	86 (50 %)	43 (25 %)	43 (25 %)
Expectation on 9 : 3 : 4 ratio	*96·75*	*32·25*	*43·0*
Expectation on 1 : 2 : 1 ratio	*86*	*43*	*43*

The figures fit perfectly a 1 : 2 : 1 ratio of agouti, steel and black, and diverge considerably from the 9 : 3 : 4 ratio. The sex of every rabbit was not recorded, but as the ratio of males to females in the large majority which were determined showed nothing abnormal, the sexes were omitted from the tables. The steels were for the most part self-coloured, but, as is usual, a few animals showed traces of the white Dutch markings.

The following hypothesis, which, as Professor Punnett pointed out, makes the case parallel with that of his "agouti-blacks[1]," appears to afford an adequate explanation of the phenomena. An agouti-black is a black rabbit with a slight development of agouti hairs at the nape of the neck. The main feature in the case of the agouti-blacks was the existence of a factor *D*, which deepened the melanic pigment. It behaved differently, according to whether it was present in the homozygous or the heterozygous condition. If heterozygous, it would turn an agouti into an agouti-black; if homozygous, it converted an agouti into a full black, which in appearance was indistinguishable from a normal black.

In the case of the steel rabbits, the factor comparable to *D* may be called *X*. Like *D* it appears to have a darkening effect, but to a less degree. It is a darkening factor which (1) in the heterozygous condition turns agouti into steel; and (2) in the homozygous condition converts agouti into black. Thus, if *A* is the factor for agouti and *a* the factor for black, then:

$AAxx$ = agouti,

$AAXx$ = steel,

$AAXX$ = black extracted from steels,

$aaxx$ = normal black.

In other words "one dose" of *X* turns agouti into steel, and "two doses" of *X* turn agouti into black. This obviously means that there are two forms of black, which are the same in appearance but genetically quite different. These two forms may be represented as already shown. Moreover, an animal cannot be steel or agouti unless it is at least heterozygous for *A*.

From these considerations it follows that:

(1) Steel × steel will, as has already been shown, give the 1 : 2 : 1 ratio of a heterozygous form.

(2) Black (extracted from steels) having the constitution $AAXX$, mated to normal agouti ($AAxx$), will give nothing but steel ($AAXx$).

[1] Punnett, R. C., *Journal of Genetics*, Vol. II. p. 227, 1912.

(3) Steel ($AAXx$) mated to agouti ($AAxx$) will give equal numbers of steel and agouti.

(4) Finally, black (extracted from steels) having the constitution $AAXX$ mated to steel ($AAXx$) should give equal numbers of steel and black.

These matings were accordingly made with the following results:

TABLE II. *Black* (extracted from steel) × *Agouti* (homozygous).

Mating ♀ × ♂	Steel	Agouti	Black
66 *c* × 51 *b*	5	—	—
77 × ,,	3	—	—
77 × ,,	6	—	—
77 × ,,	2	—	—
77 × ,,	7	—	—
78 × ,,	5	—	—
78 × ,,	6	—	—
78 × ,,	4	—	—
83 *a* × ,,	6	—	—
84 *a* × ,,	5	—	—
91 × ,,	5	—	—
91 × ,,	6	—	—
92 × ,,	6	—	—
92 × ,,	5	—	—
101 × ,,	6	—	—
65 *c* × 45 *b*	6	—	—
57 *c* × 109	5	—	—
Total	88	—	—

Most of these matings were made with the ♂ 51 *b*, kindly given by Professor Punnett, a homozygous agouti whose ancestry was known for a number of generations. The male parents in the last two matings of the table were agoutis extracted from steels. These agoutis were presumably of the supposed composition $AAxx$, since they gave nothing but steel. It appears, however, as will be shown later, that more than one type of agouti can exist. The same male, 51 *b*, when mated to steels, produced, as was expected, equal numbers of steel and agouti.

TABLE III. *Steel* × *Agouti* (homozygous).

Mating ♀ × ♂	Steel	Agouti	Black
11 × 51 *a*	3	3	—
11 × ,,	4	2	—
25 × ,,	3	5	—
54 *a* × ,,	3	3	—
56 *a* × ,,	1	2	—
55 *b* × 51 *b*	1	—	—
55 *b* × ,,	2	1	—
44 *b* × ,,	4	3	—
79 × ,,	3	1	—
38 *a* × ,,	2	3	—
60 *a* × 45 *b*	4	—	—
68 *b* × 85 *a*	3	2	—
Totals	33 (57 %)	25 (43 %)	—
Expectation	*29*	*29*	—

The agouti ♂ 51 *a* used for some of these matings also came from Professor Punnett, and was believed to be of the same breeding as the agouti 51 *b*. With both males the numbers of steel and agouti were approximately equal. In the last two matings the agouti parents were extracted from steels, 68 *b* being a ♀ and 45 *b* a ♂.

TABLE IV.

Black (extracted from steels) × *Steel.*

Mating ♀ × ♂	Steel	Agouti	Black
66 *c* × 85 *a*	—	—	1
66 *c* × 86 *a*	3	—	1
73 *b* × 70 *a*	1	—	3
73 *b* × 38 *a*	1	—	3
77 × 86 *a*	1	—	2
84 *a* × ,,	2	—	2
Totals	8 (40 %)	—	12 (60 %)
Expectation	*10*	—	*10*

The figures in Table IV diverge rather widely from the expected ratio, but the number of animals bred was small. Otherwise it will be seen that all the four suppositions on pp. 93–94 have been fulfilled with sufficient accuracy.

A number of matings were also made in order to test the question whether steels which were made by mating blacks (extracted from steels) to agoutis (Table II), behaved in the same way as the original steels. The following table shows that this was the case.

TABLE V.

Steels (from black × agouti) mated *inter se.*

Mating ♀ × ♂	Steel	Agouti	Black
50 *b* × 97	—	2	1
50 *b* × ,,	1	3	—
50 *b* × ,,	1	1	1
103 *a* × 67 *c*	2	1	—
106 *a* × ,,	4	—	—
106 *a* × ,,	5	—	—
107 *a* × ,,	4	1	—
115 × 118	2	1	3
115 × ,,	2	—	1
115 × ,,	2	3	2
116 × ,,	2	1	—
116 × ,,	2	1	2
116 × ,,	3	2	1
116 × ,,	3	1	—
117 × ,,	1	3	2
117 × ,,	3	—	3
Totals	37 (51 %)	20 (27 %)	16 (22 %)
Expectation	*36·5*	*18·25*	*18·25*

These proportions are nearly the same as those in Table I, and need no further comment.

If normal blacks are expressed by the formula *aaxx*, and blacks extracted from steels by the formula *AAXX*, as has been suggested, an interesting result should follow the mating of these two forms of black with each other. All the offspring of such matings ($aaxx \times AAXX$) should have the composition *AaXx*, and must therefore all be steel. As the result of an experiment, in which a strain of homozygous chocolates was used in place of the normal blacks, the following families were bred:

TABLE VI.

Black (extracted from steels) × *Chocolate.*

Mating ♀ × ♂	Steel	Agouti	Black
57 *c* × 31 *a*	2	—	—
57 *c* × 31 *a*	3	—	—
57 *c* × 31 *a*	4	—	—
56 *c* × 73 *b*	3	—	—
66 *c* × 31 *b*	4	—	—
84 *a* × 31 *b*	3	—	—
Total	19	—	—

In all matings but one of the above table, a chocolate was used as the male parent. In the mating 56 *c* × 73 *b*, the female parent was a chocolate. The fact that two blacks to all outward appearance exactly the same (but genetically different) should produce nothing but steel offspring, seems to be explicable on some such hypothesis as that already outlined.

Since the families in Table VI were bred, some of the same blacks have been mated to blues, i.e. blacks carrying the dilution factor. In these three litters the black ♀♀ 66 *c* and 84 *a* and the black ♂ 73 *b* gave 7 steel and 4 black young. An explanation of this unexpected result can only be looked for in the constitution of the blues. Thus it is possible that the blues used were heterozygous for Punnett's *D* factor, in which case we should expect equal numbers of animals with the constitution *AaXxdd* (= steel) and *AaXxDd*. An animal of the constitution *AaxxDd* is an agouti-black, i.e. not far removed from a full black in appearance, and on the addition of a further dose of *D* such an animal becomes a full black. It seems not unlikely that the addition of a dose of *X* to an animal heterozygous for *D* may have the effect of producing the full black coat.

As was said above, mating together the two forms of black produces steels as in Table VI, having the composition *AaXx*. The result of

pairing two of the latter individuals has not yet been ascertained, although the matings are now being effected. It is clear, however, that as the four gametes are AX, Ax, aX, ax, when these meet, sixteen rabbits will be produced consisting of black, steel and agouti, in the ratio 7 : 6 : 3. This assumes of course, as previously stated, that to be steel an animal carrying X must also be at least heterozygous for A—thus $aaXx$ will be black. In an exactly analogous manner Professor Punnett's[1] agouti-blacks of the constitution $DdEEAa$ produced black, agouti-black and agouti in the same ratio of 7 : 6 : 3.

It was mentioned on p. 94 that more than one type of agouti might exist, because a certain agouti male did not behave in the same manner as the others, when mated to blacks of the constitution $AAXX$. The explanation of this anomalous behaviour has not been discovered, but the work is being continued in the hope that some light may be thrown on the matter.

From Table II it will be seen that blacks of the form $AAXX$ mated to homozygous agoutis produced, as was expected, nothing but steel. This male, however, 51*a*, bred by Professor Punnett in the same way as the ♂ 51*b*, gave in addition to steels a number of agoutis, as follows:

TABLE VII.

Black (extracted from steel) × *Agouti* ♂ 51 *a*.

♀	Steel	Agouti	Black
46 *a*	5	2	—
46 *a*	—	6	—
47 *a*	3	4	—
48 *a*	2	4	—
48 *a*	2	4	—
Totals	12	20	—

The figures are very small, but it appears as if an equal number of steel and agouti might have been expected. At the same time it must be carefully noticed that this ♂ 51*a*, when mated to steels, behaved in exactly the same way as the ♂ 51*b*, which produced equal numbers of steel and agouti (see Table III). Unfortunately, until another agouti can be found which behaves in the same way as ♂ 51*a*, the question must remain unsolved. So far, the few animals tested have behaved quite normally.

As this is going to press, a paper by Pap[2] has been received which reviews most of the work on the coat-colour of rabbits, and contributes

[1] *Loc. cit.*

[2] Pap, Endre. *Zs. f. induktiv. Abstammungs- und Vererbungslehre*, XXVI. p. 185, 1921.

certain new facts. The author says that some of his unpublished experiments confirmed in every particular the work of Punnett on the extension and deepening factors *D* and *E*, and he gives data which show that in agreement with Punnett[1], these two factors are completely linked. Punnett observed that the factor *B* which converts the chocolate series into the black, had an interesting effect upon his agouti-blacks. If instead of an ordinary agouti-black (*DdEEBB*) he produced a similar animal heterozygous for *B*, the coat would have far more of the wild character. In fact, so far as the back is concerned, the excellent figure in Punnett's Plate[2] is not at all unlike a steel. Such rabbits were bred by Pap, and to them he gives the name of "*eisengrau*", which he says is the term used by breeders of Flemish Giants to denote this particular colour. The bellies of Pap's rabbits often had much of the wild character, but could always be distinguished from those of true wild rabbits. This I understand is also the case with Flemish steels.

The main point of interest is that Pap says rabbits carrying *D* and *E*, and also homozygous for *B*, which should be full black, can still be modified by another factor to *eisengrau*. These *eisengrau* are heterozygous for the dilution factor, which converts black into blue and chocolate into lilac. In fact, all Pap's rabbits with *D* and *E*, which should have been agouti-black or full black, varied from a dark form of agouti, through *eisengrau*, to a form not quite so dark as agouti-black. This divergence from Punnett's results was proved, it is said in a note at the end of Pap's paper, to be due to the fact that all these rabbits were heterozygous for the dilution factor.

It is very difficult to give an opinion as to the relation of these *eisengrau* rabbits to the steel of the present paper, but it seems doubtful whether Pap was dealing with rabbits of the same constitution. In the first place, so far as is known, all the animals in the author's experiments were in the black and not the chocolate series; and secondly, as was said on p. 91, special care was taken to remove the dilution factor from the strain used. It is of course possible that Pap may be mistaken in the interpretation of his results, which may have been due to an extension factor similar to Punnett's *D*, or to the one described in this paper, and not, as he thought, to the dilution factor.

[1] Punnett, R. C. *Journal of Genetics*, Vol. v. No. 1, p. 37, July, 1915.
[2] Punnett, R. C. *Journal of Genetics*, Vol. II. 1912. Plate XII, fig. 1.

CONCLUSIONS.

1. The peculiar ticking which causes the coat pattern known as "steel" in Dutch rabbits may be represented as a heterozygous character.

2. The factor for this character, called X, may be considered as a darkening or melanising factor, similar to D in the "agouti-blacks" of Punnett, but rather weaker in its effect. In a heterozygous condition, it converts an agouti rabbit into a steel; and in a homozygous condition, it converts an agouti into a black.

3. The data show that blacks extracted from steels, and therefore homozygous for X, when mated to agoutis (homozygous for a) give nothing but steel; and moreover, the same blacks when mated to normal blacks or chocolates ($aaxx$) give nothing but steel.

4. There is some evidence to show that there may be more than one type of agouti, for two agouti males were used, which when mated to steels gave equal numbers of steel and agouti, but when mated to extracted blacks, one gave nothing but steel, the other both steel and agouti. On this point, further investigations are being made.

I should like to thank Professor Punnett for his help and encouragement throughout the experiments, and Mr W. Auton for his management of the rabbits in extremely difficult circumstances.