A REVISED MAP OF THE FIFTH CHROMOSOME OF THE DOMESTIC RABBIT

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I. INTRODUCTION

CASTLE (1926) described a three-point backcross experiment in the rabbit involving the linked factors for Dutch spotting, English spotting, and Angora hair. He also considered that the three genes are located on the fifth chromosome of the rabbit in that order.

The Dutch pattern in rabbits is caused by a gene p which is almost completely recessive to self-colour P. The amount of white in this type of rabbit is also influenced by at least three minor recessive genes which hitherto are known to recombine freely with each other.

English spotting En is an incompletely dominant mutation which finds its expression in a complete inhibition of pigment formation in a considerable part of the coat. Its dominance is also influenced by the presence or absence of minor modifiers.

Angora hair l is the result of the increased length of the hair fibre found in certain breeds of rabbits. It behaves as a recessive to normal short hair.

The present work is undertaken as an attempt to present accurate recombination fractions between the three genes and to correct the hitherto known mapping of the fifth chromosome of the domestic rabbit in the light of a re-study and a re-calculation of the data presented by Castle (1924-1926).

2. DUTCH-ANGORA LINKAGE

In undertaking a comprehensive study of linkage in rabbits, Castle found that the Dutch and Angora genes are linked together with a recombination fraction of 14·26 per cent. The evidence for this linkage is summarised in table 1.

TABLE 1

Double backcross data for (Dutch-Angora) linkage. Data from Castle

Nature of cross	$++$	$p+$	$+l$	pl	Total
Coupling . . .	97	20	13	72	202
Repulsion . . .	88	433	438	52	1011
					1213

Since the difference between the linkage values of the two sexes is not significant, all the data were combined together with the tri-coupling backcross data summarised in table 5.

TABLE 2

Combined data for (Dutch-Angora) linkage

Nature of cross	$++$	$p+$	$+l$	pl	Total
Coupling . . .	330	54	42	313	739
Repulsion . . .	88	433	438	52	1011
					1750

Now we have a grand total of 1750 young of which 236 are cross-overs. This gives a recombination fraction of 13·48571 per cent.

3. ENGLISH-ANGORA LINKAGE

Castle also showed that the genes for English spotting and Angora are linked with a recombination fraction of 13·59 per cent. The evidence for this linkage is summarised in table 3.

TABLE 3

Double backcross data for (English-Angora) linkage.   Data from Castle

Nature of cross	$En+$	Enl	$++$	$+l$	Total
Coupling . . .	602	90	98	617	1407
Repulsion . . .	14	64	65	10	153
					1560

In connection with the original data of Castle, it should be noted that the matings designated as repulsion should be considered as coupling since all the heterozygous parents were of the constitution

TABLE 4

Combined data for (English-Angora) linkage

Nature of cross	$En+$	Enl	$++$	$+l$	Total
Coupling . . .	835	120	132	857	1944
Repulsion . . .	14	64	65	10	153
					2097

$En+/+l$, and those designated as coupling should be considered as repulsion since the heterozygous parents were of the constitution $Enl/++$. Evidently the use of the terms coupling and repulsion

has been reversed. Coupling signifies that the two dominants are located in one chromosome and the two recessives in the other, irrespective of whether one or both mutants are dominant.

Here again the difference between the linkage values in the two sexes is not significant and all the data were, therefore, combined together with the tri-coupling backcross data summarised in table 5.

In a grand total of 2097 young there are 276 crossovers or 13·16166 per cent. recombination fraction.

4. ENGLISH-DUTCH LINKAGE

It appears now that the two spotting genes p and En are linked since both are linked with Angora with almost equal recombination fractions in each case. This indicates either that the genes for Dutch and English are close to the same locus or that they are on opposite sides of Angora and about equally distant from it. Castle determined

TABLE 5

Tri-coupling backcross data for (English-Dutch-Angora) linkage.
Data from Castle

$$En+ +/+pl \times +pl/+pl$$

Short Dutch	Angora Dutch	Short English	Angora English	Angora Dutch English	Total
34	240	233	29	1	537

the order by the introduction of Angora into a cross involving English and Dutch. Short-haired English rabbits have been crossed with triple recessives, *i.e.* long-haired Dutch, resulting in the production of triple heterozygotes $En+ +/+pl$. Tri-coupling backcrosses were then made and produced as a rule equal numbers of English and Dutch individuals. In addition, very rare crossovers may also be produced. These of course are of two kinds, *viz.* English-Dutch and self-coloured animals lacking both the Dutch and English factors. So far, Castle has obtained only one example of the first. Because of the rarity of crossovers Castle concluded that the two spotting genes are very closely linked, and located on the same side of Angora with a recombination fraction of 0·186 per cent. The evidence for this is summarised in table 5.

These data indicate that there is one crossover in a total number of 537 rabbits or 0·186 per cent. recombination fraction.

Castle says " Linkage tests English-Angora and Angora-Dutch, indicate that English is nearer to Angora than Dutch is, since the crossover percentage in the former case is 13·0±0·8 and in the latter 14·2±0·9. The data of table 17A (table 5 in this paper) indicate

the same relation. . . . This evidence is particularly convincing because it is based on matings in which both relations are simultaneously being tested ".

Now, examining the entire data of all the linkages we find that the data on Dutch-Angora give 236 recombinations out of 1750 or 13·48571 per cent. ; the data on English-Angora give 276 recombinations out of 2097 or 13·16166 per cent. So far as these data go they suggest that the Dutch gene is further from Angora than the English gene ; but this indication is very slight.

The χ^2 test of significance in this case is that of the two by two table.

TABLE 6

Two by two table

	Combinations	Recombinations	Totals
Dutch . . .	1514	236	1750
English . . .	1821	276	2097
Totals . .	3335	512	3847

Using Yates' correction for continuity we have for $\chi_c{}^2$ a value of 0·06100698 only, whence χ_c is 0·2469959. The probability of observing a χ value as great or greater than 0·247, appropriate to the hypothesis that the locus for English is in the middle is 0·4025. On the opposite hypothesis that Dutch occupies the middle locus, we need the probability of observing a disproportion less than that observed or in the opposite direction. Consequently Yates' correction is reversed. χ_c now comes to 0·342315 and the probability to about 0·63394. The odds are, therefore, in the ratio 0·63394 to 0·4025 in favour of English lying in the middle.

But in the mating $En++/+pl \times +pl/+pl$ the only observed crossover between Dutch and English was Dutch-English-Angora, showing that the locus for English had in this case separated from the two loci for Dutch and Angora ; if English were in the middle locus, this would be a double crossover whereas if Dutch were in the middle locus it would be a single crossover only.

Now, if we accept Kosambi's formula for the relation between the recombination fraction and the map distance it appears that if y_1 and y_2 are the recombination fractions in the two segments then the frequency of recombination in both these segments is :

$$\frac{2y_1 y_2 (y_1 + y_2)}{1 + 4 y_1 y_2}$$

so that of those gametes showing recombination in the second

segment the fraction which also show recombination in the first must be :

$$\frac{2y_1(y_1+y_2)}{1+4y_1y_2}$$

If now, as in our present problem, y_2 is exceedingly small, this fraction is reduced to $2y_1^2$.

Taking the two loci together there have been in all 512 recombinations with Angora out of 3847 or 13·30907 per cent. for y. Hence $2y_1^2$ is 0·035426 or 1/28·2, hence single crossovers are nearly 29·2 times as frequent as double crossovers among gametes showing the crossover between the English locus and the Dutch.

Combining the two probabilities we have calculated, we see that the combined event of a single crossover with a sampling deviation exceeding 0·247 of the standard error is about 18·3 times more probable than the combined event of a double crossover with a deviation exceeding −0·342.

In consequence of these calculations I am inclined to believe that the Dutch gene lies in the middle between the English gene and the Angora gene. The recombination fractions on this hypothesis will be as follows :

Angora-Dutch	.	.	13·216 per cent.
Dutch-English	.	.	0·186 „
Angora-English	.	.	13·402 „

As we have determined the right order of the three genes for Angora, Dutch, and English, it remains to find the accurate recombination fractions between them.

Since such problems, in this as in many other cases, lead to troublesome algebraic expressions, it is useful to apply the arithmetical approach supplied by *Fisher's Scoring System* (1946). As in all iteration processes of approximation the precision required in working out the consequences of the trial values adopted is difficult to foresee, it is, therefore, usually convenient to carry out the work to the highest accuracy readily obtainable ; *e.g.* to use ten significant figures if a ten figure machine is employed. This course was followed in the work set out below, in which, however, the numerical values have been cut down before publication usually to seven significant figures, since it appears from the results arrived at that this number would have been sufficient.

Now, if we score y_1 and y_2 at the trial values 13·23 and 0·186 per cent. respectively we must therefore score y_{12} at

$$\frac{y_1+y_2}{1+4y_1y_2} = 13·40281 \text{ per cent.}$$

moreover

$$\frac{dy_{12}}{dy_1} = \frac{1-4y_2^2}{(1+4y_1y_2)^2} = 0·9980205$$

$$\frac{dy_{12}}{dy_2} = \frac{1-4y_1^2}{(1+4y_1y_2)^2} = 0·9281587$$

At 13·23 per cent. recombination fraction the score for y_1 (Angora-Dutch) will be :

$$
\begin{array}{lll}
173/y & 173/0\cdot1323 & -1307\cdot634 \\
1040/(1-y) & 1040/0\cdot8677 & +1198\cdot571 \\
\hline
& & -\;\;109\cdot063
\end{array}
$$

and the amount of information will be :

$$1213/(y)(1-y) \quad 1213/(0\cdot1323)(0\cdot8677) = 10566\cdot51.$$

At 13·40281 recombination fraction the score for y_2 (Angora-English) will be :

$$
\begin{array}{lll}
212/y & 212/0\cdot1340281 & -1581\cdot75816 \\
1348/(1-y) & 1348/0\cdot8659719 & +1556\cdot63245 \\
\hline
& & -25\cdot12571
\end{array}
$$

and the amount of information will be :

$$1560/(y)(1-y) \quad 1560/(0\cdot1340281)(0\cdot8659719) = 13440\cdot80$$

Hence

	Factor	Score	Information
For Angora-Dutch . .	0·9980205	−25·07597	13387·64
For English-Dutch . .	0·9281587	−23·32065	11579·00

The geometric mean for the two values equals :

$$(13440\cdot80)(0\cdot9980205)(0\cdot9281587) = 12450\cdot50$$

In the case of the tri-coupling data, the expected frequencies of the four gametic types produced by the triple heterozygote could be expressed in terms of y_1 and y_2 only as represented in (table a). The total frequency is $1+4y_1y_2$ of which the two fractions, *i.e.* the original combinations and the recombinations, gives frequencies in those classes $(1-y_1)(1+4y_1y_2)$ gives old combinations for y_1 and $y_1(1+4y_1y_2)$ gives the recombinations for the same segment.

Similarly, fraction $1-y_2$ and y_2 of the total give frequencies for the segment y_2. Contingency table, simultaneous crossovers in both segments are represented by the following :

$$\tfrac{1}{2}\left(y_1+y_2 - \frac{y_1+y_2}{1+4y_1y_2}\right) = 2y_1y_2(y_1+y_2)$$

since the frequency $2y_1y_2(y_1+y_2)$ is involved twice in a sum of total number of crossovers for both segments, *i.e.* y_1+y_2.

The other two tables (b and c) are constructed for $\dfrac{d}{dy}$ with respect to y_1 and y_2 respectively.

TABLE a

Frequencies of the four gametic types produced by the triple heterozygote

		y_2	
		Old combinations	New combinations	
y_1 { Old combinations	$1-y_1-y_2+2y_1y_2(2-y_1-y_2)$	$y_2-y_1y_2(y_1-y_2)$	$(1-y_1)(1+4y_1y_2)$
New combinations	$y_1+2y_1y_2(y_1-y_2)$	$2y_1y_2(y_1+y_2)$	$y_1(1+4y_1y_2)$
	$(1-y_2)(1+4y_1y_2)$	$y_2(1+y_1y_2)$	$1+4y_1y_2$

TABLE b

Differential coefficient with respect to y_1

$1-4y_1+4y_1y_2+2y_2^2$	$4y_1y_2-2y_2^2$	$1+8y_1y_2-4y_2$
$-1-4y_1y_2+2y_2^2$	$-2y_2(2y_1+y_2)$	$-1-8y_1y_2$
$-4y_2+4y_2^2$	$4y_2^2$	$4y_2$

TABLE c

Differential coefficient with respect to y_2

$1-4y_1+4y_1y_2+2y_1^2$	$-1-4y_1y_2+2y_1^2$	$-4y_1+4y_1^2$
$+4y_1y_2-2y_1^2$	$-2y_1(2y_2+y_1)$	$-4y_1^2$
$1+8y_1y_2-4y_1$	$-1-8y_1y_2$	$4y_1$

Now considering that the values for y_1 and y_2 are 0·1323 and 0·00186 respectively, the three tables may be represented after substitution as follows :

TABLE a′

0·8667583	0·0017958	0·8685541
0·1323642	0·0000660	0·1324302
0·9991225	0·0018618	1·0009843

TABLE b′

+0·9935512	+0·0009774	+0·9945286
−1·0009774	−0·0009912	−1·0019686
−0·0074262	−0·0000138	−0·0074400

TABLE c′

+0·5067909	−0·9659777	−0·4591868
−0·0340223	−0·0359909	−0·0700132
+0·4727686	−1·0019686	−0·5282000

The score equals $\dfrac{dm}{dy}/m$, but m is equal to a/A where A is equal to $1+4y_1y_2$ and a is equal to each fraction of the expectations. Therefore, $\log m = \log a - \log A$. Hence $\dfrac{dm}{dy} \times 1/m = 1/a \times da/dy - 1/A \times dA/dy$ and this will give the score.

Referring to the tri-coupling data illustrated in table 5, we find that the old combinations and the new combinations of the two segments, *i.e.* y_1 and y_2 are as follows :

		y_2		
		Old combinations	New combinations	
y_1 { Old combinations . .		473	1	474
{ New combinations . .		63	0	63
		536	1	537

The scores for y_1 are :

$$+1·153717 \quad +0·5517025$$
$$-7·554865 \quad -15·0107492$$

The scores for y_2 are :

$$+1·1133765 \quad -537·38072$$
$$+0·2716441 \quad -544·78798$$

The total score for $y_1 = +70·30335$.

The total score for $y_2 = +6·35994$.

The amount of information for the two segments is obtained in the form of a matrix consisting of i_{11}, i_{12}, i_{22}.

For i_{11} the amount of information equals :

$$m(1/m \times dm/dy_1)^2 = 1/A\{1/a(da/dy_1)^2 - 1/A(dA/dy_1)^2\}.$$

For i_{12} the amount of information equals :

$$m(1/m \times dm/dy_1)(1/m \times dm/dy_2) \; \ldots$$

the product of the two informations

$$= 1/A\{1/a(da/dy_1)(da/dy_2) - 1/A(dA/dy_1)(dA/dy_2)\}.$$

For i_{22} the amount of information equals :

$$m(1/m \times dm/dy_2)^2 = 1/A\{1/a(da/dy_2)^2 - 1/A(dA/dy_2)^2\}$$

Information for y_1	Information for y_{12}	Information for y_2
1·138892	0·5809263	0·2963
7·756969	0·2572865	0·0874
0·000532	−0·5257479	519·6076
0·014880	0·5403088	19·6182
−0·000005	−0·0039334	−0·2798
8·911268	0·8488403	539·3297
4780·645	455·37900	289335·3

Now the whole data and calculations should be combined as follows for comparison :

| Test | Rabbits | Scores | | i_{11} | i_{12} | i_{22} |
		A–D	D–E			
Angora-Dutch .	1213	−109·06300	...	10566·51	...	...
Angora-English.	1560	−25·07597	−23·32065	13387·64	12450·50	11579·00
Tri-coupling .	735	+70·30335	+6·35664	4780·64	455·38	289335·30
		−63·83562	−16·96401	28734·79	12905·88	300914·30

With these values we can write the equations :

$$28734\cdot79\, dy_1 + 12905\cdot88\, dy_2 = +63\cdot83562$$
$$12905\cdot88\, dy_1 + 300914\cdot30\, dy_2 = +16\cdot96401$$

where dy_1 and dy_2 stand for the adjustments required in the recombination fractions adopted. The solution of these equations is :

$$dy_1 = +0\cdot0022394 \quad +0\cdot22394 \text{ per cent.}$$
$$dy_2 = -0\cdot0000397 \quad -0\cdot00397 \text{ per cent.}$$

The accurate recombination fractions for y_1 and y_2 are 13·454 and 0·182 per cent. respectively. Using Kosambi's formula, the recombination fraction for y_{12} will be 13·623 per cent.

The map lengths calculated from the recombination fractions obtained (using table VII, Fisher and Yates) are given in the summary.

5. SUMMARY

A correction of the hitherto known mapping of the fifth chromosome of the domestic rabbit has been made in the light of a re-study and a re-calculation of the data presented by Castle (1924-1926).

The new recombination fractions and the corresponding map distances between the three genes involved are as follows :—

	Recombination fraction per cent.	Map distance
Angora-Dutch	13·454	13·794 centimorgans
Dutch-English	0·182	0·182 ,,
Angora-English	13·623	13·976 ,,

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