Cuénot Lucien, " The heredity of pigmentation in mice (5th note)", *Archives de zoologie Experimental and General ,* 1907, ^{4th} series , 6 , p. I-XIII.

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In previous notes (1st , 2nd and 4th notes, 1902-1905) , I have published the results of experiments made on different races of mice, notably the gray (wild type); black, yellow and albinos; I showed that one could define each of these races, from the point of view of the coloring of the coat and the eyes, by a certain constitution of the germinative plasm, and I designated by letters the specific determinants which the latter contains . The set of these letters, for a given race, constitutes the hereditary formula of this one; knowledge of these formulas and of the relative dominance of the various mutations of the same determinant

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makes it possible to calculate, by applying the rules of Mendelian heredity, the result of the most complicated crosses.

I continued this research by studying two new races, less simple than the previous ones: Pigmented mice with red eyes, and brown mice.

Pigmented red-eyed mouse

Darbishire paradox

DARBISHIRE (1905) **,** having crossed Mice of fawn coat but with red eyes, by albinos, also with red eyes, obtained only Mice with black eyes, generally of gray coat (340 pups, all with black eyes): this result Surprisingly enough, even paradoxically, the Tawny Mice, as well as the albinos, have themselves had red-eyed parents for as long as one would like to suppose. And yet, the immediate offspring of the cross have perfectly black eyes.

These hybrids, crossed between them, have a very complicated offspring, including on the one hand albinos, on the other hand pigmented forms, some with red eyes (coat sometimes fawn [*fawn* or *yellow*], sometimes pearl gray [*lilac*] , the others with black eyes (grey, black, yellow coat, etc.) The relative proportion of these three categories is as follows (out of 555 pups):

137 albinos: 24.7%.
134 pigmented with red eyes: 24.1%.
284 pigmented black-eyed: 51.2%.

PERSONAL EXPERIENCES . - I do not intend, in this note, to criticize DARBISHIRE 'S WORK IN DETAIL ; I will content myself with saying that he obtained complicated results because he used albinos of very different value from the point of view of latent characteristics of coloration. To avoid this pitfall, I started with a single couple, comprising a red-eyed fawn male, bought in England, and an albino female, descended from black ancestors, and whose hereditary formula I knew by virtue of her origin and previous trials (AN). This unique pair gave me 6 little ones, all with black eyes and having the same coat: the coat is a slightly reddish gray, the belly is white edged with red, which is exactly the livery of the field mouse (*Mus sylvaticus* L.).

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These 6 hybrids were crossed with each other, and had the following offspring (92 offspring):

21 albinos

25 pigmented with red eyes: 22 fawn and 3 pearl gray.

46 pigmented black-eyed: 36 white-bellied gray and 10 black.

INTERPRETATION . - I searched for a long time for a rational explanation of the DARBISHIRE PARADOX , crossing in all directions the first generation hybrids and their descendants; the experiments are made rather difficult by the extremely delicate health of the fawns and the pearl grays, which often die at a young age, or produce only an insignificant number of young. Nevertheless, I ended up finding a very simple solution to the problem, completely in line with Mendelian doctrines.

The formula of the red-eyed fawn mouse, the original father, is GG'E..., that is to say that its particularities are related to at least three germplasm determinants: C is the determinant common to all pigmented breeds: G' and E react on each other to give the tawny color of the coat, E being at the same time a special determinant of the non-pigmentation of the eyes.

The formula of the albino used as the original female is ANM...: A is the special determinant of albinism, common to all albinos, which prevents N and M from expressing themselves; N is the determinant of black, when it is in company with C and M; M is at the same time the special determinant of the pigmentation of the eyes, when it is in the presence of C ; but I repeat that N and M have no effect here, since A prevents all pigmentation.

In short, it is a cross between two races each defined by three symmetrical determinants, C opposing A, G' to N and M to E. It suffices to know that C is dominant over A, G' over N, and M over E, and we have all we need to solve the DARBISHIRE PARADOX .

The offspring resulting from the cross between the two red-eyed breeds are therefore trihybrids , having the formula C(A)G'(N)M(E), the dominated determinants being placed in parentheses; or for

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abbreviate , they are somatically identical, to a CG'M form. The eyes are black, since there is a union of the determinants C and M; the animal is gray with a white belly, the result of the reaction of G' and M in the presence of C.

The second generation, resulting from the crossing of trihybrids , must be broken down as follows (1) , by applying the well-known rules of Mendelian disjunction and dominance:

Out of 64 pups:

16 albinos (A...), i.e. 25%;

12 pigmented forms with red eyes, i.e. 18.75%, including 9 with fawn coat (CG'E...) and 3 forming a new combination (CNE).

36 pigmented forms with black eyes, i.e. 56.25%, including 27 gray with white belly (CG' M...) and 9 black (CNM...)

The new combination (CNE), predicted by the theory, is evidently that which gives rise to the pearl gray mice with red eyes, a race which appears suddenly in the offspring of the trihybrids , and which differs both from these and from all their known ancestors.

Let us now calculate, according to the above, the theoretical forecast out of 92, the number of babies that I obtained:

FORECAST _ _ THEORY : _ _

23 albinos;
17 including 13 fawn and 4 pearl gray;
52 including 39 gray with white belly and 13 black.

RESULT _ _ REAL : _ _

21 albinos;
25 including 22 fawn and 3 pearl gray;
46 including 36 white-bellied gray and 10 black.

The predicted numbers in my hypothesis and the real ones are really- [really]

(1) In the table, I only give the overall result of the calculation, indicating only the determinants which are expressed in the coloring of the coat and the eyes.

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lie very close to each other; there are only a few too many fawns, a particularity which is also found in the DARBISHIRE CROSSES , and which we will try to explain later.

Naturally, I continued the demonstration in detail, by verifying the hereditary formulas attributed to the so-called crossbreeding products of the trihybrids . I will confine myself to quoting some of the results, which always agree with my hypothesis:

1° If we cross a red-eyed fawn (CG'E) that is clearly homozygous, with a pearl gray (CNE), we should only obtain fawns, since G' is dominant over N; this is indeed what happens, as DARBISHIRE HAD ALREADY SEEN and as I checked after him.

2° The pearl gray with red eyes is the pigmented form which contains the most dominated determinants (N and E): consequently, pearl gray crossed between them must give only pearl gray and nothing else. This is what I found: a pair of pearl grays, currently under observation, produced 17 young, all similar to the parents.

3° The black race (CNM) and the pearl gray race (CNE) differ only by a single determinant; the result of their crossing is therefore a monohybrid , which must be black, since M is dominant over E. These black monohybrids , crossed with each other in turn, must produce black-eyed blacks and red-eyed pearl grays, in the proportion of 3 to 1. This is exactly what happens from the color point of view, but I don't have enough staves yet to verify the numerical proportion.

4° The theory predicts the existence of albinos containing the determinant E; these, crossed with fawn or pearl gray with red eyes, which contain the same determinant, must therefore produce little ones with red eyes, and not black as in the paradoxical cross at the beginning. I have indeed met such albinos.

Once convinced of the validity of the explanatory hypothesis, I began other research by crossing fawn and pearl gray red-eyed mice with gray, yellow and brown mice. They are not sufficiently advanced for me to be able to account for them; I will however mention that the CG'M group (pelage similar to that of *Mus sylvaticus*) is dominant over the CGM group (Ordinary gray mouse, *Mus musculus*) but on the other hand , that the CJM group (Yellow mouse), dominates GG' as well M than CGM.

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brown mice

Brown mice (*chocolate , brown , plum* des Anglais) are a breed easily recognizable by their coat of a beautiful velvety brown, a little lighter under the belly; the eyes are black; the tail has a mixed shade between pink and brown; the hairs contain only brown pigment, excluding black and yellow. Some authors, PARSONS (cited in BATESON 1903) **,** ALLEN (1904) and DAVENPORT (1904) used for certain crosses this brown race or the corresponding albino, but their experiments lack precision and it is impossible to deduce from them the formula of germinal constitution. So I had to resume this study, starting with a pair of brown mice which had been obligingly sent to me by Mr. DARBISHIRE .

EXPERIENCES PERSONAL . - I first crossed brown mice with each other, then with the different pure breeds that I have, the gray (CG), the gray with a white belly (CG'), the black (CN) and the yellow (CJ ...).

Browns, crossed between them, always and only give browns similar to the parents (so far 47 young); unless, of course, these mice contain the special determinant of albinism, in which case we obtain browns and albinos. This result, in agreement with those of PARSONS , ALLEN , and DAVENPORT , enables us to foresee that the brown is a race dominated by all the others, and that any brown mouse whatever is necessarily purebred.

Indeed, when the brown race is crossed by another, gray, black or yellow, the hybrids are never brown.

1) Brown X Gray = Gray
2) Brown X White-bellied Gray = White-bellied Gray
3) Brown X Black = Black
4) Brown X Yellow (constantly heterozygous form) = Yellow + Gray or Black.

Let us study in particular the hybrids of cross no. 1. If they are monohybrids , by crossing them together, we will obtain, in accordance with the rules of Mendelian disjunction and dominance:

Grey-Brown Hybrid X Grey-Brown Hybrid = 3 Gray + 1 Brown.

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However, this does not say everything that happens; the actual result is much more complicated. The products of the cross belong to four different types:

1° and 2° Grays and browns, similar to the grandparents; result that was expected.

3° Typical blacks, which is quite unexpected.

4° A new form, which corresponds to the *cinnamom - agouti* of the English (*golden - agouti* of ALLEN); the coat is mixed between gray and brown; its color is yellowish-brown or golden gray, and is distinguished with the greatest facility from that of the grandparents; the hairs contain brown and yellow pigment, but no black pigment.

The grays are in the great majority; the blacks and the golden-agouti are less numerous; finally, browns appear quite rarely. I obtained 76 babies which are distributed as follows:

41 gray, 15 black, 15 golden-agouti, 5 brown.

INTERPRETATION - AFTER having crossed in all possible ways the products of the crossing of the hybrids and thus checking the results reported by the authors quoted above, I arrived at a completely satisfactory interpretation.

We have seen that the previous cross gives, among other things, black mice, which absolutely do not exist in the ancestral line of the gray and brown parents.

The appearance of black is due to a contribution of specific determinants of this race (CN), contribution for which the brown strain is necessarily responsible. The brown mice, like the black ones, therefore contain the CN group, but since they are not similar, there must therefore be between these two races one or more differential determinants, unknown up to now. In reality, there is only one: I will call F (first letter of the word *dark*) the determinant in question as it exists in black mice and D (first letter of the word *diluted*) its mutation in Brown mice. The black formula therefore becomes CNF, and the brown one CND. D is dominated by F, as can be seen from the crosses between blacks and browns.

If we also attribute to gray mice (CG) this new determinant F, everything is then very easily explained: the original cross gives rise to dihybrids , the parents differing by the determinants GN, on the one hand, FD, on the other go. Here is the forecast calculation based on the previous assumptions:

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[Table not reproduced in this version]

The new CGD combination obviously corresponds to the new form (golden-brown = golden-agouti), which appeared suddenly during the crossing of the gray dihybrids , and which differs both from these and from all their known ancestors.

Let us now calculate, according to the above, the theoretical forecast out of 76, the number of babies that I obtained:

FORECAST _ _ THEORY : _ _

43 gray; 14 black; 14 golden agouti; 5 brown

RESULT _ _ REAL : _ _

41 gray; 15 black; 15 golden agouti; 5 brown

The agreement between forecast and reality is so striking that there is no reason to doubt the accuracy of the hypothesis put forward above. I have, moreover, carried out all sorts of verifications, which have always given the results which one can foresee by the handling of hereditary formulas.

The study of pigmented mice with red eyes and that of brown mice has therefore revealed the existence of two new determinants of coat color: the determinant M presenting the mutation E, and the determinant F presenting the mutation D. complete formula, until now, of a Gray Mouse, becomes CGFM; that

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a black mouse, CNFM; that of a brown mouse, CNDM; that of a red-eyed tawny mouse, CG'FE; that of a pearl gray mouse with red eyes, CNFE, etc. We can propose the following verification, which I have not yet tried, for lack of available material, but of which I announce the result in advance: if we cross a pearl gray mouse with red eyes, although homozygous, with a brown, also homozygous, we will have to obtain Black Mice, and just that:

CNFE X CNDM = CNFM (CNDE)

Notion of unit characters

Experience has proved that among the various transmissible characters, there are some which are absolutely independent of other characters, and which are, at least temporarily, indecomposable. Thus, in mice, the property of waltzing and variegation are characters entirely independent of the color of the coat; by suitable crosses, they can be transferred to all possible Mice, white, grey, black, yellow, etc. In white mice, the variegation is naturally not visible, since the very bottom of the coat is white; but they are able to transmit the character to their descendants, just like the mice with pigmented and variegated coats. The indecomposable particularities which are thus inherited, in a separate and independent manner, are the *elementary characters* or *character-units* ; to each of them corresponds in the germinal plasm a special substance or *determinant* , susceptible of variation or of independent mutation.

It is of the utmost importance not to confuse the unit-character with the *descriptive character* , as understood in a definition of animal or plant; a word suffices to say that a Mouse is black, that the petals of a Poppy are red, or that a Wallflower is hairy; now, these simple descriptive characters can very well correspond to several independent character-units, which only well-directed crosses can bring to light. I was, I believe, the first to show that the color of the coat of mice comprised several character-units; I now know four of them, and it is probable that there were others ; Bateson, Saunders and Punnet (1905) think hairiness

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of the *Matthiola* corresponds to four character-units which they denote by letters (HKCR).

On the other hand, there are complicated descriptive characters, including many details; which appear to correspond to a single character-unit, for example the variegation of the Mouse: the location and shape of the white areas are regulated by factors which undoubtedly arise during ontogenesis, such as a distribution of nerves, vessels or adipose panniculus, so that it is impossible to assure the hereditary transmission of this or that detail; what is transmitted is simply a certain quantitative value of variegation.

I will cite yet another very characteristic example: we know that the gray coloration of wild mice is due to the juxtaposition of hairs colored by various pigments; a black, a brown and a yellow, without counting the white or absence of pigment; it would be quite natural to think that each of these four hues has its special determinant; the races of simple color, black, brown, yellow or white, would possess only the *ad hoc determinant,* and in the gray race alone all the determinants would coexist. Experiments have abundantly proved that things are quite different; there are indeed several determinants for the color of the coat, but there are the same number in the unicolored races and in the gray race; these races differ, not in the quantity of their determinants, but in the quality; a simple color is not the result of the predominance of a special determinant, but the resultant of the mutual reaction of several determinants.

When several determinants of germplasm correspond to a descriptive character, these can of course only be detected insofar as they have undergone mutations. Suppose, for example, a coat color that consists of five character-units, CGMFU; if there is only one, determinant C, which has presented an A mutation over time, when the CGMFU race is crossed with the AGMFU race, everything will happen as if the coloration had a single determinant, CA; it will be quite impossible, and moreover perfectly useless from a practical point of view, to highlight the four other determinants common to the two races. We cannot therefore count in an *absolute way* all the character-units corresponding to a descriptive character, but the more the species studied presents different varieties, the more we will have

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chances that the mutations have affected a large number of character-units, without it being ever certain to know them all.

It is because of not having known the profound distinction to be established between descriptive character and character-unity that all research on heredity based on the interpretation of statistics, with whatever mathematical apparatus they may be surrounded, have given only approximate or even perfectly inaccurate results; it is for the same reason that experiments that are a little hasty or lacking in rigor, such as those of breeders, provide incomprehensible or capricious results, from which no precise rule can be deduced. Hybridization experiments only manage to be perfectly clear when it has been possible, by a delicate analysis, to define the character-units involved.

Findings.

The color of the coat, in mice, is represented in the germplasm by a certain number of determinants (character-units), which experience alone allows to highlight and count; so far, we know at least 5 of them, and it is very likely that there are still others. Each of these determinants can present independent mutations.

These determinants, designated by letters, are as follows:

1. It is a determinant of color in general; it exists in all more or less pigmented mice. It presents the mutation A which corresponds to the absolute deprivation of color (albinism), whatever the determinants which accompany it.

2. M, when it accompanies C, is the determinant of the black color of the eyes and influences the general tint by making it darker; it has the E mutation, which corresponds to the red coloring of the eyes and influences the general tint by lightening it.

3. G is a special determinant of coat color in the presence of C; it has a large number of mutations: G', N, and J.

4. F is a determinant which contributes with the preceding ones to give the color of the coat; it presents the D mutation, the action of which results in the disappearance of the black pigment in the hairs.

5. U is the determinant of the uniform coloring of the coat, whatever its hue; it presents the P mutation, with a series of variants, $p^1, p^2, p^3... p^n$, which correspond to varying degrees of variegation.

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It is to the cooperation of the determinants C, ME, GG'-NJ, FD and to their reaction one upon the other that the special tints of the different races of mice are due.

The following list indicates the known combinations, as well as those still unknown, but possible (the 5th determinant, uniform coat or variegation, is not taken into account, the action of which is manifested by the absence or presence of white on the colored background).

CGFM = Ordinary gray coloring (wild gray mouse), more or less dark, due to the mixture of three pigments: black, yellow and brown, and white, non-pigmented hairs.

CG'FM = Gray color on the back, reddish white under the belly (very similar to that of the Field Mouse [*Mus sylvaticus* L.]).

CNFM = Black, due to the mixture of black and brown pigments.

CJFM.... = More or less dark yellow, but can only exist in the combined state in heterozygotes.

CGDM = Disappearance of the black pigment of the hairs, the animal is of a yellowish brown (golden gray).

CG'DM = Like the previous one, except that the belly is rufous white.

CNDM = Brown.

CJDM=?

CGFE = Fawn coat (dirty yellow); dark red eyes, almost garnet in color.

CG'FE = More or less light fawn coat; light red eyes.

CNFE = Pearl gray coat; light red eyes.

CJFE = bright yellow coat; light red eyes.

CGDE, CG'DE, CNDE, CJDE = Unknown.

AGFM, AG'FM, ANFM, AJFM..., AGDM, etc... = Albinos.

The rules of dominance of the various mutations of the same determinant are summarized in the following table; a mutation

given dominates those which are placed below, in a vertical line, and is dominated by those which are above:

[Table not reproduced in this version]

The list of the result of the combinations and the table of dominance make it possible to predict and calculate the results of all the possible crosses. And conversely, given the results of crosses, they make it possible to indicate which are the probable parents.

All the determinants known in mice, i.e. those mentioned above, those of uniform variegation-dress (UP) and those of waltz and rectilinear locomotion (RW), *strictly follow the rules of l Mendelian inheritance. We only know of mice Mendelian characters.*

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