TRICOLOR INHERITANCE. I. THE TRICOLOR SERIES IN GUINEA-PIGS[1]

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TABLE OF CONTENTS

INTRODUCTION

In 1897 FRANCIS GALTON published a paper on color inheritance in the Basset hound. This breed of dog has two color varieties, the "tricolor," an animal with black, tan (also called lemon) and white patches, and the "lemon-and-white," with tan (or lemon) and white patches. Neither of these varieties seemed to breed true. Lemon-and-whites might come from tricolored parents, and they might also have tricolor offspring. The pedigrees of 817 dogs furnished the material upon which the paper was based. GALTON applied his "law of ancestral inheritance," proposed some years before (GALTON (1889), to his data, and found that there was a close resemblance between the expected numbers and those actually

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obtained. GALTON's law of ancestral inheritance as stated by himself (GALTON 1897, p. 402) is as follows:

"The two parents contribute between them on the average one-half, or (0.5) of the total heritage of the offspring; the four grandparents one-quarter, or $(0.5)^2$; the eight great-grandparents, one-eighth, or $(0.5)^3$, and so on. Thus the sum of the ancestral contributions is expressed by the series $[(0.5) + (0.5)^2 + (0.5)^3$, etc.], which, being equal to 1, accounts for the whole heritage."

These results have long been a stumbling-block to those who have tried to put a Mendelian interpretation on all forms of color inheritance. Several attempts have been made to attack the problem by working with guinea-pigs. In 1912 CASTLE published the results of his experiments on tricolor inheritance in these animals. He says (CASTLE 1912, p. 438):

"The tricolor animal is white marked with irregular but distinct blotches of black and yellow. Tricolors produce, besides tricolors, young which are black-and-white or yellow-and-white, but never in my experience those which are wholly free from white. In other words they breed true to spotting with white, but not to spotting with black and yellow. The black-and-white as well as the yellow-and-white offspring of tricolor parents may produce tricolor young. Indeed any of these three conditions is able to produce both the others. Notwithstanding the fact that *neither the black-and-whites nor the yellow-and-whites produced by tricolors breed true,*[2] there are races of black-and-white and of yellow-and-white guinea-pigs which do breed true. It remains to explain why the others do not. A black-and-white animal which breeds true may be considered to possess some chemical substance necessary for the production of color (which we call a color factor) distributed irregularly throughout its coat. Wherever this substance is wanting no color is formed and a white area results. The specific factor for black (probably an enzyme) is however everywhere present in the coat, so that wherever color forms, the color is black. Such races as this breed true.

"The yellow-and-white animal which breeds true may likewise be considered to have an irregularly distributed color factor, but to lack entirely in its coat the black factor. Hence the color, wherever formed, is yellow.

"Yellow races also exist which do not bear spots of white, but which have spots of black. In such animals [commonly known as tortoises] the color factor is evidently uniform in distribution, whereas the black factor is irregularly distributed.

"Now the tricolor race is a yellow one spotted both with white and with black, i.e., it results from irregularity in distribution through the coat of two different chemical substances, the color factor and the black factor. These two factors are known to be independent of each other in heredity. See CASTLE (1909). It is therefore not to be supposed that they will coincide in distribution. If the black factor extends over all the colored areas, the animal will be black-and-white. If the black factor falls only on areas which lack the color factor, it will produce no visible effect, and the animal will be yellow-and-white. If, finally, the black factor falls on some

[2] Original not italicized.

of the colored areas, but not on all of them, those in which it falls will be black, the others yellow, and the uncolored areas of course white. Hence a tricolor will result. But the gametic composition of these tricolors will not be different from that of the black-and-whites, or red-and-whites, produced by the same race, since all alike will be characterized by irregularity in distribution of the same two factors. A tricolor race on this hypothesis should be unfixable, as has up to the present time been found to be true."

Although CASTLE was able to pick out the different characters concerned and to show their relationship to each other, his results show that a finer analysis is necessary in order to prove that we are dealing with Mendelian allelomorphs. In his table giving his results he takes for granted that all tricolors are alike and accordingly gives the offspring from such matings with their percentage of tricolors. He treats the red-and-whites and black-and-whites in a similar manner. It is the object of the present paper to demonstrate that there are two kinds of tricolors, three kinds of red-and-whites and two kinds of black-and-whites. By dividing the three color varieties into these different classes it is possible to show relatively good Mendelian ratios in the offspring.

GOODALE and MORGAN (1913) have also published a paper on the inheritance of the tricolor coat in guinea-pigs. They followed methods similar to those employed by CASTLE, but in addition mated some of their tricolors to self ("uniform") reds and self blacks. They made the observation that when the agouti factor is present in a tricolor, the black spots are always affected and as a result we have an animal with white, red and agouti spots.[3] They objected to CASTLE's hypothesis of overlapping areas as an explanation for the formation of the three different color varieties, because it seemed very improbable to them that mere chance would account for the proportionately large numbers of red-and whites, and black-and-whites of tricolor parentage. In their concluding paragraph they state,

"Our matings show that the distributer for black is dominant, as seen in tricolor by uniform, and tortoise [black-and-red] by uniform, giving tortoise, and tricolor by tricolor giving bicolor black [black-and-white]. On this hypothesis our original race of tricolors must have been heterozygous for the black distributer, and hence could throw some bicolor blacks which are real bicolors, not overlapped bicolors. This explains our excess of bicolor black which belonged to both types."

From the foregoing paragraphs it is clear that the great difficulty in an analysis of the guinea-pigs in what may be termed the tricolor series (comprising black-and-whites and red-and-whites of tricolor parentage

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[3] The same thing is true of the chocolate spots, which become "cinnamon-agouti." This follows from the fact that the agouti factor produces a visible effect on black and chocolate pigment, but not on red.

as well as tricolors themselves) lay in the masking of the real relation of black to red by the more or less complete overlapping of these areas by white. In other words every animal in the tricolor series is in reality a tortoise in which the black-red pattern is partially obscured by the white markings. Ordinarily the white covers only a part of the black and a part of the red, and the result is a tricolor; if all the red areas are completely overlapped it is a black-and-white; if the black happens to be completely covered a red-and-white results. It should be remembered that in each case identically the same factors are concerned, the difference in appearance depending altogether on the distribution of the red and black pigmented areas and the non-pigmented (white) areas. It is apparent that if the white could be eliminated the study of the black-red relationship would be greatly facilitated. This result can be accomplished indirectly by mating the animals of the tricolor series to homozygous self reds, as a consequence of which all the offspring will be entirely pigmented and the distribution of red and black can be observed directly. Breeding to self reds constitutes therefore the best test of the real composition of any animal spotted with white, and is the method upon which we have based our analysis in the present investigation. As a result we have found, as has been said, that when the facts are fully analyzed the various matings of the different color varieties result in ordinarily good Mendelian ratios.

Before proceeding further, it seems desirable to name and describe accurately the factors involved. At the outset it may be stated that there are no new factors to name or describe, all of them having been designated by CASTLE in previous papers. In some cases it has seemed advisable to modify or to amplify the description of these factors, and in three cases to employ different symbols for them.

FACTORIAL ANALYSIS

Factors always present

When we leave out of consideration some of the newer, and therefore uncommon, color varieties recently described by CASTLE (1914), we may say that there are two factors which are always present in all color varieties of the guinea-pig. These are R, the factor for red, and C, the factor for chocolate or brown.[4] When unmodified by other factors, red is found

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[4] CASTLE uses C as the symbol for the color factor and Br as the symbol for brown or chocolate. As there is an obvious advantage in having only one letter to represent a factor, it was thought advisable to use P (pigmentation) as the symbol for the factor for color and C as the symbol for the factor for chocolate or brown.

throughout the skin and coat of the entire animal, while chocolate, under like conditions, is restricted to the eyes, eyelids, and the skin of the ears and feet. Every self red animal, therefore, must carry chocolate. Since both these factors are always present they are commonly assumed, and accordingly left out, in writing the factorial composition of the animals.

Other factors

B is the factor for black. Like C, the factor for chocolate, it is restricted to the eyes, eyelids, and to the skin of the ears and feet when unmodified by other factors. Black is epistatic to chocolate.

S represents the factor for *self* pigmentation, meaning that the entire coat is pigmented,—there are no white spots. It is allelomorphic to spotting with white. Tortoises, although not uniformly pigmented, must, nevertheless, carry the factor S; they may however be heterozygous for it (Ss), in which case, when inbred, they throw some offspring spotted with white (ss), as was demonstrated by GOODALE and MORGAN (1913). Although S and its absence are treated as simple Mendelian allelomorphs in the present paper, evidence is accumulating which seems to indicate that the relationship is not as simple as has hitherto been supposed. It will not, however, vitiate results to consider S at this time as a simple dominant to its absence.

E is the extension factor. When present it extends the black or the chocolate throughout the coat, giving a uniform black or a uniform chocolate. In the absence of S, as a result of which the animal is white spotted, pigment formation fails where the white spots occur and we therefore have black or chocolate animals with white spots. Black-and-whites or chocolate-and-whites, homozygous for E, will necessarily breed true.

E' was used by CASTLE (1909) for a factor which extends the black or the chocolate pigment only partially through the coat, thereby forming relatively large black or chocolate patches. He also mentions an E'' factor which causes the black or the chocolate to be extended in small numerous spots. None of the animals we have worked with seem to carry this latter factor. E is dominant over E', as was mentioned by LITTLE (1913, p. 42), and from facts now at hand, and which will be published soon, it is evident that these factors stand in the relation of allelomorphs to each other, e being recessive to both. In order to conform more closely with the terminology used by T. H. MORGAN and his associates, in other cases of multiple allelomorphs, it has been deemed advisable to use the symbol e^p in place of E', the "p" in this combination referring to a lessened and therefore partial degree of "extension", the

resulting character being spotting with chocolate or black on a red background. In the presence of S and in the absence of E, an animal carrying c^b is therefore a tortoise, i.e., a red animal with black or chocolate patches. In the absence of S under the same conditions we have a tricolor. Both Ee^b and Ee, in the presence of S give uniform self black (when B is present) or uniform chocolate (in the absence of B). It is not improbable that the type with smaller and more numerous spots referred to above will be found to fall in this same series of multiple allelomorphs, but this has not as yet been determined.

The accompanying table (table 1) shows concisely the various color types produced by the different possible combinations of the factors which have been defined in the foregoing paragraphs, and reference to it will facilitate the discussion which is to follow. It may be of interest to note that all the types mentioned have been produced in our laboratory. Figure 1 represents diagrammatically the color relations of the types carrying B (black). These diagrams would do equally well for the chocolate series (b), since in the absence of B the chocolate would occupy exactly the black areas.

<center>EXPERIMENTAL RESULTS</center>

In the present discussion it may greatly simplify matters when tabulating results to make no distinction between black and chocolate. Animals of the formula $e^b e^b$ or $e^b e$ are spotted with melanic pigment, either chocolate or black. The spots are of chief interest.

The results obtained from the various matings will be given in the following pages. We shall first attempt to show that there are two kinds of tricolors, (1) those homozygous and (2) those heterozygous for e^b, the black-spotting factor.

Tricolor × self red

Formula No. 7 (see table 1) is that of a tricolor. It is evident that two kinds of tricolor zygotes may be formed, i.e., one that is homozygous for e^b, and another kind that is heterozygous for the same factor, viz., $e^b e$. Both kinds have been obtained. The method employed in testing them has been to mate them with uniform reds. In the former case all the offspring should be tortoise, while in the latter we should expect approximately half tortoises and half self reds, as follows:

Tricolor (type 7), $e^b e^b ssBB$ × self red (type 10), $eeSSbb$ = tortoise (type 5), $e^b eSsBb$;

Tricolor, $c^b essBB$ × self red, $eeSSbb$ = 1 tortoise, $e^b eSsBb$: 1 self red, $eeSsBb$.

TABLE I

Constant factors	Allelomorphic series	Alternative allelomorphs		Type No.	Phenotypic formula	Phenotypic appearance
		S (*complete pigmentation*)	*B* (*black present*)	1	(*RC*)*ESB*	= self black
			b (*black absent*)	2	(*RC*)*ESb*	= self chocolate
	E (*complete extension of melanic pigment*)	*s* (*spotting with white*)	*B* (*black present*)	3	(*RC*)*EsB*	= black-and-white
			b (*black absent*)	4	(*RC*)*Esb*	= chocolate-and-white
		S (*complete pigmentation*)	*B* (*black present*)	5	(*RC*)*eᵖSB*	= tortoise (black-and-red)
			b (*black absent*)	6	(*RC*)*eᵖSb*	= tortoise (chocolate-and-red)
R (*red*) *C* (*chocolate*)	*eᵖ* (*partial extension; spotting with black or chocolate*)	*s* (*spotting with white*)	*B* (*black present*)	7	(*RC*)*eᵖsB*	= tricolor (white-black-and-red)[a]
			b (*black absent*)	8	(*RC*)*eᵖsb*	= tricolor (white-chocolate-and-red)[a]
		S (*complete pigmentation*)	*B* (*black present*)	9	(*RC*)*eSB*	= black-eyed self red
			b (*black absent*)	10	(*RC*)*eSb*	= brown-eyed self red
	e (*non-extension of melanic pigment*)	*s* (*spotting with white*)	*B* (*black present*)	11	(*RC*)*esB*	= black-eyed red-and-white
			b (*black absent*)	12	(*RC*)*esb*	= brown-eyed red-and-white

[a] Under certain conditions these types may appear as red-and-white or as black (or chocolate)-and-white. See diagrams, figure I, types 7 *b* and 7 *c*

Type 1	Type 3'	Type 3''	Type 3'''
Self black (ESB)			
	Black-and-whites (EsB)		

Type 5	Type 7a	Type 7b	Type 7c
Tortoise (e^pSB	Tricolor	Red-and-white	Black-and-white
	Tricolor Series (e^psB)		

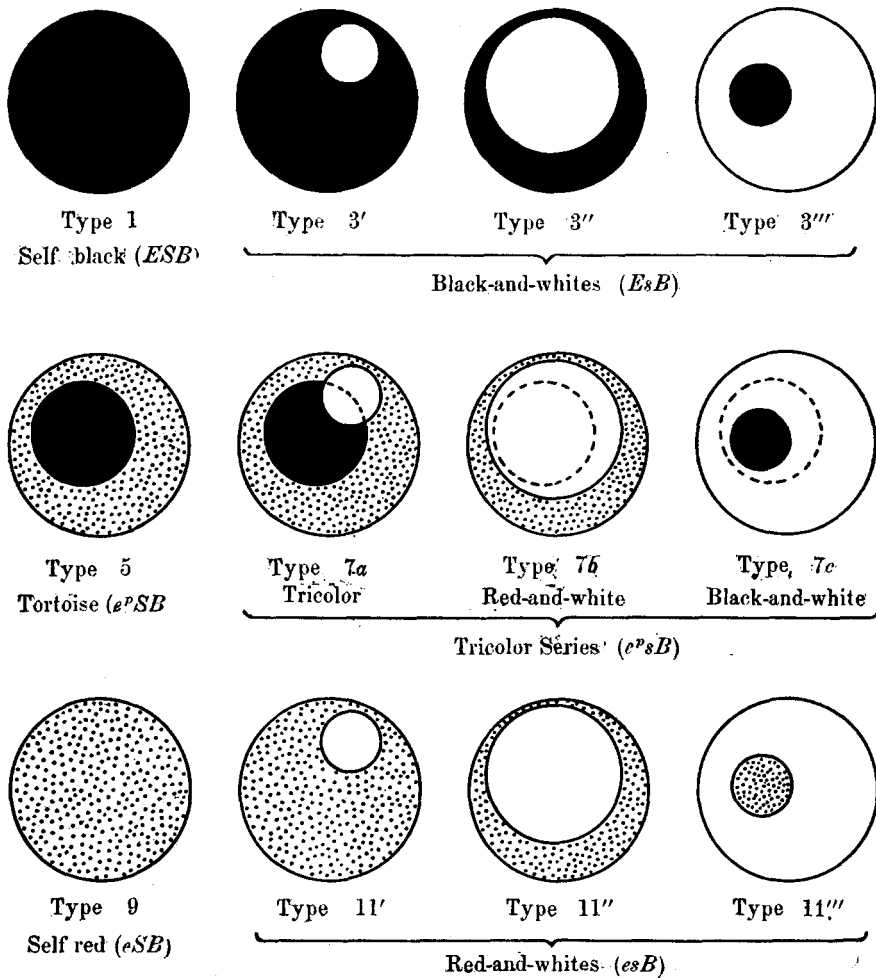| Type 9 | Type 11' | Type 11'' | Type 11''' |
| Self red (eSB) | | | |
| | Red-and-whites (esB) | | |

FIGURE 1.—Diagrammatic representation of the color types carrying B shown in table 1. Red is represented by dotted areas, black by solid color, and white is left unshaded. In 7 a, 7 b and 7 c the dotted line represents the outline of the black spot shown in type 5. The diagrams in type 7 illustrate how the three appearances, tricolor, red-and-white and black-and-white, may depend entirely upon the distribution of the white. The pairs, 3''' and 7 c, and 11'' and 7 b indicate how two animals may be phenotypically alike but genotypically different. Type numbers are the same as in table 1.

Table 2 shows the actual results of the breeding tests. The 81 offspring from the mating homozygous tricolor × self red represent litters from 8 tricolor individuals.

TABLE 2

Tricolors × self reds

Parents	Offspring		
	Tortoise (incl. tricolors)	Self red (incl. *ee* red-and-whites)	Red-and-white (not tested)
Self red × tricolor ($c^p c^p$)	81	0	0
Self red × tricolor ($c^p c$)	37	47	9

This means an average of 10 offspring to each tricolor tested. In no case was the number of offspring for any individual less than 5[6], while in two instances it was 15. Four of the homozygous tricolors were mated to other tricolors but this kind of mating will be discussed later.

Some of the self reds used in the heterozygous tricolor × self red cross were not homozygous for self (S) and hence some white spotted offspring resulted. The tricolor and tortoise offspring have been lumped together as "tortoises" because we know they all carry the c^p factor. The red-and-whites, however, may or may not carry the c^p factor and have therefore been classified separately. If those matings in which red-and-whites occur among the offspring were excluded, the numbers would be 33 tortoises and 43 self reds. Whether or not the red-and-whites are eliminated it will be noted that the self reds are in either case considerably in excess. A similar excess of extracted reds has been noted by CASTLE (1905) in guinea-pigs and by COLE (1914) in pigeons.

Tortoise crosses

Some of the tortoise offspring obtained from the tricolor × self red crosses described above were mated together. The zygotic composition of these animals would be $c^p c S s$. We should therefore expect 9 tortoises ($c^p S$) : 3 self reds ($c S$) : 3 tricolors ($c^p s$) : 1 red-and-white ($c s$) in the offspring. The tricolors differ from the tortoises and the *ee* red-and-whites differ from the self reds only in that they show white spotting ($s s$). White spotting, however, does not behave as a simple Mendelian recessive. In the first place, when a homozygous self animal is crossed with a white-spotted one we have in no case obtained complete dominance

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[6] Five was chosen arbitrarily as a number giving a fair indication of homozygosity. A larger number would have been desirable, but owing to the small litters and the long gestation period in guinea-pigs it is often difficult to obtain more than five offspring from a single individual. Several tricolors, when mated to self reds, had only 1 to 4 offspring, all tortoises, but these seemed too few to establish their homozygosity, and hence the records are not included in the table.

of self in the first generation, since in all such crosses some of the F_1's have shown a small white blaze on the head or one or more white feet. When these slightly spotted F_1's are inbred they produce some offspring like themselves as well as selfs and ordinary white-spotted of the grandparental type. These same classes of offspring are obtained by inbreeding the self-colored animals of the first generation, which makes it appear that the two F_1 types are hereditarily alike.

Another puzzling phenomenon is that sometimes when two extracted white-spotted animals having comparatively large white spots are bred together they get among their offspring some animals that are selfs. From the above considerations it will be seen that there is some difficulty in distinguishing between true self animals and true recessive white-spotted ones. We have therefore in our tortoise crosses disregarded white spotting as much as possible and considered chiefly the presence or absence of e^p, the black spotting factor. When this is done the tortoises and tricolors are put in one class because they carry the e^p factor and self reds and ee red-and-whites in the other because they lack it. The expectation then would be 3 tortoises or tricolors to 1 self red or ee red-and-white.

Heterozygous tortoises (e^pe) similar to those used in the above cross were also mated to self reds. In this case the expectation is an equal number of tortoises and self reds. Here there ought not to be any offspring with large white spots if the self reds used are homozygous for self. Table 3 gives the numbers obtained and also the expected numbers for both kinds of crosses.

TABLE 3

Tortoise crosses

Parent mating		Offspring		
		Tortoise and tricolors	Self reds and *ee* red-and-whites (tested)	Red-and-whites not tested
Tortoise (e^pe) × Tortoise (e^pe)	Actual numbers	64	29	9
	Expectation	*76.5*	*25.5*	
Tortoise (e^pe) × Self red (ee)	Actual numbers	31	46	
	Expectation	*38.5*	*38.5*	

An inspection of Table 3 shows that there are two kinds of red-and-whites listed, those "tested" and those "not tested." As previously stated, red-and-whites may or may not carry the e^p factor. This point can be determined by mating these animals to self reds as will be more fully discussed later. In our $e^p e$ tortoise $\times$ $e^p e$ tortoise cross 12 red-and-whites were obtained. Of these, 9 died before they had any off-spring, or at any rate before they had enough to make certain of their composition. The above-mentioned 9 guinea-pigs constitute the red-and-whites "not tested." The remaining 3 of the 12 proved to be ee and therefore were classified with the self reds. The probabilities therefore are that the majority of the red-and-whites not tested do not carry the e^p factor, and this would tend to swell the already disproportionately large number of ee animals. As in previous crosses, the number of ee animals in the two crosses given in table 3 is considerably above expectation. Up to the present there is no good explanation for this.

It may not be out of place to make mention here of the fact that tortoises vary greatly in the extent to which their coats are made up of black (or chocolate) pigment patches. In a majority of cases these patches constitute more than half of the entire coat. There are animals, however, which have very small patches of black (see figure 3, f). On the other hand there are a considerable number that are entirely black except for a few small red spots (see figure 3, b). In 3 instances there have been animals born of tortoise parentage that to all appearance were self black. Two of these died young, while the third when mated to self reds gave birth only to tortoises and to self reds. This leads one to the natural conclusion that these "self" blacks are not in reality selfs, but only tortoises in which the black happened to be extended so far that all of the red was covered by it. The fewness of their numbers also points to the same conclusion. Following this same line of reasoning it seems probable that tortoises may be obtained in which the black is so little ex-tended that they are to all appearances *self reds*. None of these have been detected so far, although many extracted reds from tortoise crosses have been bred together.[7]

A consideration of the data given in the above paragraph would make it seem that the e^p (or black spotting) factor is a favorable one for

[7] GOODALE and MORGAN mated together self reds from a tricolor $\times$ self red cross and obtained among the offspring one animal showing a small black spot on its back. This would make it seem that at least one of the parents carried the e^p factor although it was not visibly expressed. WHITING (1915) has given an explanation similar to the one mentioned in the text, to account for the occurrence of self black female cats instead of the expected tortoiseshells in the black $♀$ $\times$ orange $♂$ mating.

testing the effects of selection on a variable character, and experiments along these lines are now in progress.

Red-and-whites

, It is obvious that three kinds of tricolor crosses can be made—one in which both the parents are homozygous for the e^p factor, another in which one parent is homozygous and the other heterozygous, and a third in which both parents are heterozygous. In the first two crosses all the offspring would have the e^p factor, though some, in the second case, would be heterozygous for it. In the third cross the expectation is that one-fourth the offspring would lack e^p entirely, i.e., would be *ee*. If any red-and-white offspring resulted from either of the first two crosses they would be sure to carry the e^p factor, while in the case of the third cross there would always be pure red-and-white offspring (*ee*) and there would also be the possibility of some red-and-whites carrying the e^p factor.

The presence or absence of the e^p factor in red-and-whites must therefore be determined by breeding tests. As has previously been explained the black spotting (e^p) when present in red-and-whites is masked because the white spotting happens to fall where the black patches would otherwise have been (see figure 1, type 7 b). To test the red-and-whites, then, it is desirable to mate them to animals that we know are *ee* and at the same time will cause the offspring to be entirely pigmented, so that if the e^p factor is present in the red-and-white it will be sure to be visibly expressed in the offspring. This result can be effected by mating the red-and-whites to homozygous self reds, which because they are *SS*, will get offspring that are entirely pigmented. If the e^p factor is present in the red-and-white being tested, all the offspring should be tortoises when it is homozygous for the factor, and half of them tortoises and the remainder self reds when it is heterozygous. All should be self reds when the factor is absent.

The above method has been used in testing all of our red-and-whites that lived long enough to be tested. We have not attempted, however, to determine whether or not a red-and-white carrying the e^p factor was homozygous for it. What we were primarily interested in was whether or not the factor was present. If five self red and no tortoise offspring were obtained it was taken as a fair indication that the animal being tested was a pure recessive (*ee*).

Summarizing those matings in which both self red and tortoise offspring were obtained, and in which the red-and-white parent was accordingly $e^p e$, gives 43 self reds and 31 tortoises instead of the expected equality. As usual the *ee* animals are in excess.

A consideration of the above paragraphs will make it apparent that three kinds of red-and-whites occur:

1. Those that are homozygous for c^b ⎫
2. Those that are heterozygous for c^b ⎬ (figure 1, type 7)
3. Those that lack it entirely (figure 1, type 11).

Black-and-whites

Black-and-whites of tricolor parentage may be variously described. For the sake of simplicity it is perhaps convenient to look upon them as tortoises (figure 1, type 5) which have had white spotting superimposed upon them in such a manner that the white areas cover up all of the red patches and probably always part of the black as well, leaving a white animal with black spots (figure 1, type 7 c). The black spotting in the above kind of black-and-whites is the same as that found in tricolors and in c^b red-and-whites (type 7 b). This can be demonstrated by matings with self reds. As before stated, $e^b e^b$ tricolors and $e^b e^b$ red-and-whites when mated to self reds have all tortoise offspring. In a similar manner one of our black-and-whites (♀ 18.1) when mated to self reds had 7 tortoise and no self red offspring. Another black-and-white (♂ 11.1) when mated to ee red-and-white females (figure 1, type 11) had offspring consisting of 6 tricolors ($e^b e$) and one red-and-white, which was tested and found to carry black spotting (therefore also $c^b e$). The results of the above matings indicate that both of the black-and-whites tested were homozygous for e^b. These animals are in the ancestry of all of our black-and-whites[8] except one, and it is probable therefore that some of the latter when tested would also have been found to be homozygous for the black spotting (e^b) factor. There is no good reason, on the other hand, for assuming that some of the black-and-whites would not have been heterozygous for the factor. If we are justified in the assumption that there are $e^b e$ black-and-whites, then we may state that black-and-whites *of tricolor parentage* are of two kinds:

1. Those homozygous for c^b ⎫
2. Those heterozygous for e^b ⎬ (figure 1, type 7 c).

No black-and-white can be ee because this would mean an animal from which the black spotting factor is absent.

A summary of the black-and-white matings is given in table 4. The fact that no ee red-and-white offspring were obtained, strongly indicates that at least one of the parents in every mating was homozygous for the black-spotting factor (e^b). This is in line with what has been stated above concerning the black-and-white animals used.

[8] By black-and-white is meant an animal in which no red spotting whatever is visible. Many of GOODALE and MORGAN's "bicolor blacks" had small areas of red in their coats.

TABLE 4

Black-and-white crosses

Parents	Offspring		
	Tricolors	Black-and-whites	Red-and-whites (e^p) (tested)
Black-and-white × Black-and-white	17	8	
Black-and-white × Tricolor	10	2	
Black-and-white × Red-and-white (*ee*)	11		1

In the first two types of matings the percentage of black-and-whites is high compared with that in tricolor × tricolor matings (see table 5). This will be taken up later in the discussion.

Tricolor × tricolor

We are now in a position to discuss tricolor × tricolor matings. As previously stated, there should be two kinds of tricolors—those that are homozygous for e^p and those that are heterozygous for it. Homozygous animals bred together should produce offspring, all of which carry the e^p factor. These would not necessarily be tricolors because black-and-whites from tricolor parents *do,* and red-and-whites *may,* also carry this factor. On the other hand tricolors heterozygous for the c^p factor should produce offspring one-fourth of which are red-and-whites that do *not* carry this factor; in addition there may be other red-and-whites which do, as is shown in table 5.

TABLE 5

Tricolors × tricolors

Parents	Offspring						Expectation	
	e^p				*ee*	Not tested	e^p	*ee*
	Tricolor	Black-and-white	Red-and-white	Total	Red-and-white	Red-and-white		
$e^p e^p \times e^p e^p$ or $e^p e$	54	2	4	60	0	2	62	0
$e^p e \times e^p e$	28	0	2	30	6	6	31.5	10.5

In the first cross recorded in table 5 at least one of the parents in each mating had been tested with self reds and had been proved to be homozygous for the black spotting factor. Therefore this factor should be present in all of the offspring. The only offspring about which there could be any doubt would be the red-and-whites. There were 6 of these, 2 of which died before they could be tested. The remaining 4 when bred to self reds proved to carry the e^p factor. It will be noted that the percentage of black-and-whites is quite low in this mating, and there are none at all in the heterozygous tricolor cross. A large number would not be expected because tricolors do not tend to carry as much black spotting as do the black-and-whites.

That both parents were $e^p e$ in the second cross recorded in table 5 was determined in one of two ways. Either they were both tested by means of matings with self reds or else it was indicated by the fact that they produced some ee red-and-white offspring. All the red-and-white offspring from parents tested in either way were mated when possible to self reds to determine whether or not they carried the black spotting factor. Six of the 14 red-and-white offspring could not be tested because of their early deaths; of the remaining 8 there were two that carried the e^p factor and six that did not.

In both crosses reported in table 5 results fairly close to expectation were obtained.

<div align="center">DISCUSSION</div>

In the present discussion we shall look upon the tricolor series in guinea-pigs as red animals irregularly spotted with black and with white. In the case of the tricolors some of the red is visible; in the black-and-whites the red is partially blotted out by the white spotting and the remainder masked by the black spotting; in the red-and-whites all of the black is blotted out by the white spotting while some of the red shows. This agrees with the explanation given by CASTLE (1912). He, however, assumes that *all* red-and-whites of tricolor parentage carry the black spotting factor, whereas we have shown that ee red-and-whites are produced as recessives when both the tricolor parents are heterozygous for e^p. Such red-and-whites breed true, contrary to CASTLE's assumption, quoted on p. 288.

Some matings tend to produce a large percentage of black-and-whites, others a fairly large number of e^p red-and-whites, and still others almost entirely tricolors. One possible explanation is that there may be factors which tend to localize and restrict the black spotting and the white spot-

ting to certain more or less definite regions. Two conditions might result, (1) if the black spots tend to fall on what would otherwise have been red areas the number of black-and-whites will be increased; (2) if the black spots tend to fall in the white areas, thus being blotted out, more red-and-whites will result. Our experience, however, substantiates CASTLE's interpretation that the black and the white spotting are entirely independent of each other and are in no sense localized, i.e., they are not any more liable to fall on one part of the body than on another.[9] Each,

a
Tortoise (with
large amount of black)   Black-and-white (e^p)   Red-and-white (e^p)

d
Tortoise (with
small amount of black)   Red-and-white (e^p)   Black-and-white (e^p)
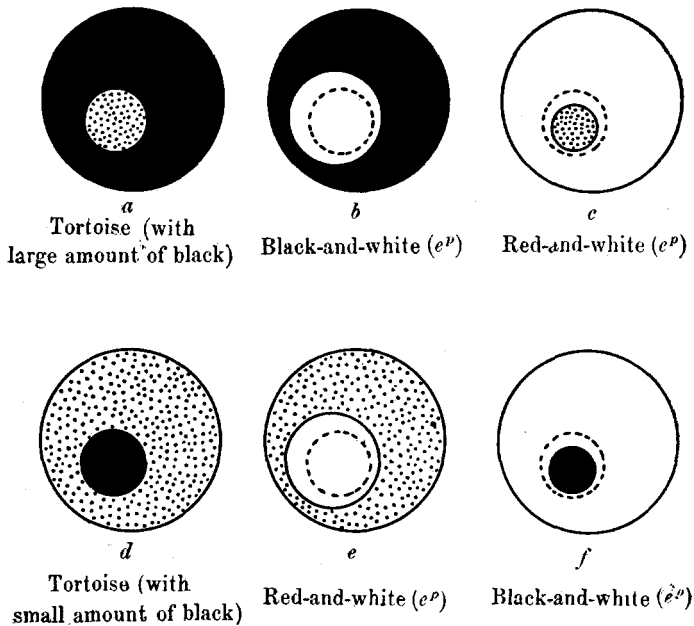
FIGURE 2.—Diagrams showing how the amount of black pigmentation in a white spotted animal may largely affect its chance of becoming a black-and-white or an e^p red-and-white. Only a small amount of white is necessary, rightly placed, to convert a into a black-and-white, as shown in b; similarly d may easily become a red-and-white (e). A great deal of white would be necessary, also rightly located (c), to make a red-and-white of a, and the same would be true to make a black-and-white (f) of d.

however, may vary in amount. This is shown by the fact that, as previously stated, the amount of black pigmentation in tortoises varies greatly, and the same may be said regarding the amount of white spotting in tricolors. It is due to these variations in the amount of black

[9] Varieties, such as the "Dutch marked," in which white seems to have been fixed in a more or less definite pattern, are not here considered. A white pattern could probably be fixed with some definiteness in tricolors, but this has never been done so far as we know.

pigmentation and the extent of the white areas that some animals which would otherwise be tricolors become black-and-whites and others c^p red-and-whites. The diagrams in figure 2 may be of service in explaining this point. They demonstrate the fact that if an animal carries a large amount of black pigmentation (a) the chances are that the red areas will be blotted out by the white spotting (b) oftener than will the black areas (c). The larger the white areas the better the chances that the red will be blotted out and that black-and-whites will result. In the same way a small amount of black pigmentation (d) and a large amount of white spotting facilitate the production of c^p red-and-whites (e), and a black-
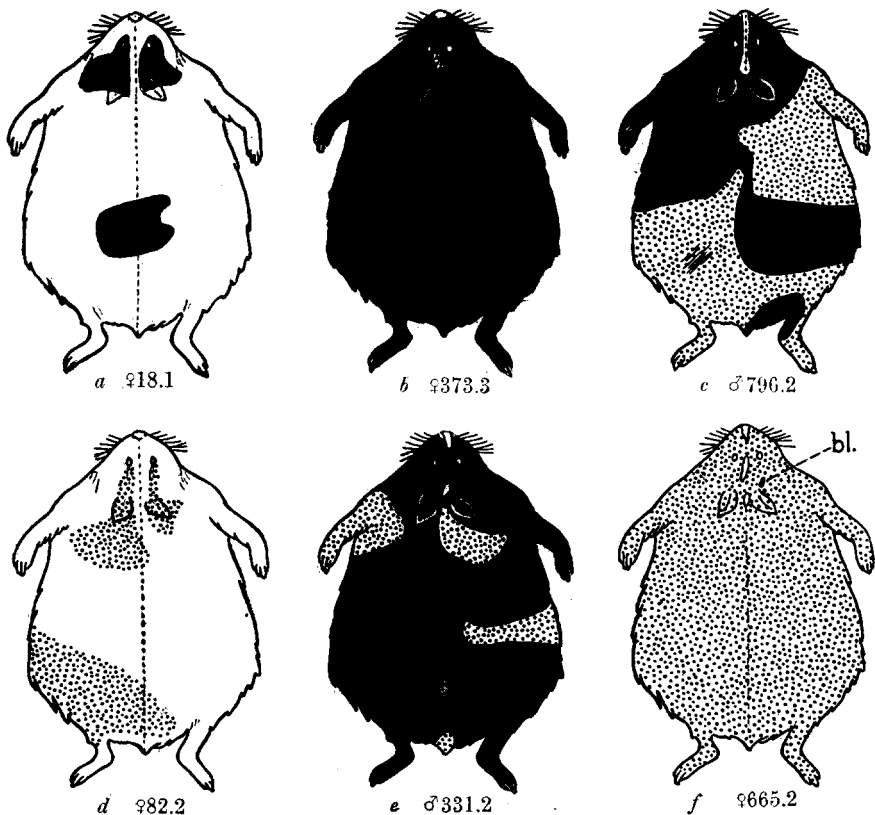

FIGURE 3.—Outline drawings of an c^p black-and-white female, a, an c^p red-and-white female, d, and two tortoise offspring of each resulting from matings with self red males. The opposite extremes of black pigmentation occurring in the tortoise offspring of a are represented by b and c. In like manner e and f represent the extremes of black pigmentation in the 8 tortoise offspring of d. (Figure 3, b is really an agouti-and-red, due to the fact that the self red male carried the agouti factor, but for the sake of simplicity it has been represented in the drawing as a black-and-red.)

and-white may be produced under these conditions when the amount of white is large enough and is properly placed (f).

What evidence have we that such is the case? In other words, can it be demonstrated that an c^b black-and-white, for instance, usually carries a large amount of black spotting and an e^b red-and-white a small amount? This cannot be determined directly by physical examination of the animals in question, but it can be approximately determined by mating the animals to be tested to self reds and thus obtaining tortoise offspring. If the tortoise offspring from a black-and-white $\times$ self red cross carry more black on the average than do the tortoise offspring from an e^b red-and-white $\times$ self red cross, we are justified in assuming that the
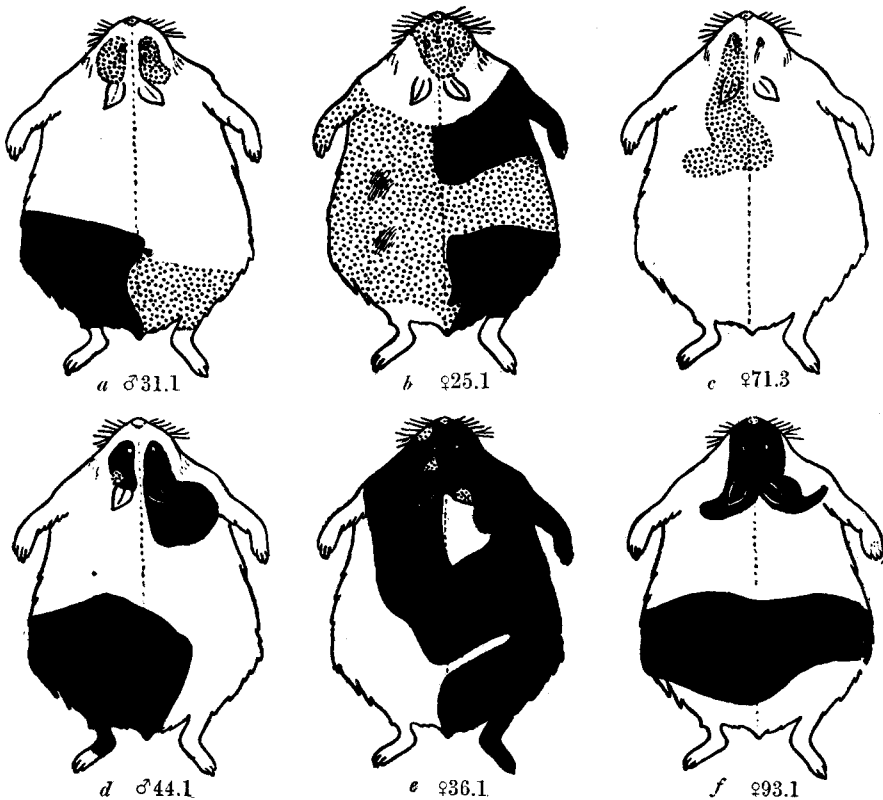

a ♂31.1 b ♀25.1 c ♀71.3

d ♂44.1 e ♀36.1 f ♀93.1

FIGURE 4.—Outline drawings of two pairs of tricolor guinea-pigs (a and b, and d and e) and one characteristic bicolor offspring of each (c and f). The pair a and b, showing a relatively small amount of black pigmentation, had 5 offspring. Of these 3 were tricolors and the other 2 were e^p red-and-whites. Diagram c represents one of the latter. The pair d and e, showing a relatively large amount of black pigmentation, had 8 offspring. Of these 7 were tricolors and one was a black-and-white. Diagram f represents the latter.

black-and-white probably carried more black pigmentation than did the c^p red-and-white. Figure 3 gives outline drawings of animals actually tested in this manner, and certain of their offspring. It is evident from a comparison of the corresponding extremes of black pigmentation in the tortoise offspring that more black occurs in the coat of the young from the black-and-white female, than in those from the red-and-white female. While we believe that this relation would hold generally, it should be mentioned that these two females are the only ones which have been sufficiently tested on this point.

If black-and-whites carry a large amount of black pigmentation it is to be expected that when the parents are tricolors these also will tend to show a large amount of black. On the other hand, tricolor parents of e^p red-and-whites should show a relatively small amount of black. Figure 4 illustrates this point with actual examples. It is noteworthy, furthermore, that tricolor parents do not as a rule produce both black-and-whites and e^p red-and-whites in addition to their tricolor offspring.

GOODALE and MORGAN (1913), in the previously quoted concluding paragraph of their paper, make the statement that tricolors throwing bicolor blacks (black-and-whites) "could throw some bicolor blacks which are real bicolors, not overlapped bicolors". By "real" bicolors they presumably mean black-and-whites which would breed true. According to our explanation such black-and-whites would of necessity carry the E or total-extension factor (figure 1, type 3). They cannot, however, obtain this factor from tricolor parents since tricolors themselves cannot carry E, which causes all pigmented areas to be black. Moreover, GOODALE and MORGAN present no proof that they did obtain such black-and-whites, but they believed there probably were "real bicolors" because it seemed to them that the large percentage of black-and-white offspring obtained in some of their tricolor matings could not be accounted for by the chance complete overlapping of all red areas by black. Their result might be sufficiently explained, however, by the fact already shown, that a large amount of black pigmentation and at the same time a large amount of white spotting are favorable for the production of black-and-whites. Furthermore some of the animals designated as bicolor blacks by GOODALE and MORGAN, showed small amounts of red and accordingly should be classified as tricolors.

CASTLE (1912) makes mention of races of red-and-whites and of black-and-whites which breed true, but he does not state how they can be produced. We have already shown (p. 300) that when $e^p e$ tricolors are mated together some ee red-and-whites are produced and these breed

true. Red-and-whites of this composition can also be produced in another manner. When self reds are mated to $e^p e$ tricolors, $e^p e$ black-and-whites, $e^p e$ red-and-whites, (i.e., animals of the tricolor series), or to ee red-and-whites, there are always some self reds produced. These self red animals must be Ss because one of their parents was white-spotted, and they are also ee since otherwise some black spotting would be visible. When they are inbred, therefore, some white-spotted offspring will result. These will breed true for the lack of black spotting, and, as a general rule, when the white spots are large, for the presence of white spotting.[10] A considerable number of red-and-whites that breed true have been obtained in this manner.

To produce black-and-whites which breed true a somewhat similar procedure is followed. In this case we want to introduce the E factor, which causes the black to be extended throughout the pigmented part of the coat. This is done by crossing a self black ($EESS$, figure 1, type 1) with an ee red-and-white. The F_1's will all be self black. Their composition will be $EeSs$. When they are inbred we should expect one out of sixteen of the offspring to have the composition $EEss$ (figure 1, type 3). These would be the black-and-whites which breed true. We have obtained animals of this kind by following the above method. One of them, ♂ 356.3, when mated to other black-and-whites had 20 offspring, all black-and-whites. When mated to ee red-and-whites, he had 18 offspring, all of which again were black-and-whites. We have neglected to mate EE black-and-whites to self reds, but we have made a mating using an Ee black-and-white. The resulting offspring consisted of 9 self blacks and 8 self reds. EE black-and-whites mated to self reds should get all self black offspring.

From the foregoing at least two methods can be derived whereby e^p black-and-whites can be distinguished from black-and-whites carrying the E factor. Inspection of the third type of mating in table 4 will show that when $e^p e^p$ black-and-whites are mated to ee red-and-whites tricolors tend to make up a majority of the offspring. In a similar kind of mating when EE black-and-whites are used, all of the offspring are black-and-whites. The above is the first method. The second method, which requires fewer offspring to determine the point at issue, consists in mating the black-and-whites to be tested to self reds. Animals homozygous for e^p produce only tortoises, while those homozygous for E produce only self blacks. It must be borne in mind, however, that we may

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[10] In a few cases we have obtained selfs when two white-spotted animals of this sort were bred together.

have animals of the formula Ec^b. These when bred to self reds should produce approximately equal numbers of self blacks and tortoises.[11]

When EE black-and-whites are crossed with c^bc^b animals of the tricolor series, the resulting offspring should be Ec^b black-and-whites. When inbred these should get the following three kinds of offspring: $1\ EE : 2\ Ec^b : 1\ c^bc^b$. The first kind would be black-and-whites which breed true; the second kind would be black-and-whites like the F_1 parents; and the third kind would be animals of the tricolor series, i.e., black-and-whites, red-and-whites or tricolors. This is the sort of mixture GOODALE and MORGAN thought they obtained from tricolor × tricolor matings. While we have not made the above cross, other work now in progress has demonstrated beyond a doubt that E, c^b and c form an allelomorphic series, which would give a result as indicated.

It may be of interest in this connection to discuss a cross made by GOODALE and MORGAN (1913, p. 332). A tricolor female when mated to a self black male produced one self black, one self red and a tortoise. She had previously been mated to a self red and had produced 7 self red offspring. Her composition would therefore be c^bc. There is nothing except the present mating by which to determine the factorial composition of the self black male. GOODALE and MORGAN conclude:

"This male appears to have been homozygous as regards lack of spotted white [SS], heterozygous for [self] black (Bb) [Ee], and also heterozygous for some factor that causes black to appear in spots [e^be], i.e., a factor analogous to the factor commonly recognized as the white spotting factor."

According to this interpretation (leaving S out of consideration) the self black male would carry our three factors, E, c^b and c. This is untenable by our hypothesis, since an individual can carry only two of the factors making up an allelomorphic series. Our interpretation is that the self black in question was Ec. We have already shown that the tricolor female is c^bc. $Ec \times c^bc = 1\ Ec^b : 1\ Ec : 1\ c^bc : 1\ cc$. This means that from the above kind of mating the expectation is 2 self blacks : 1 tortoise : 1 self red. The actual result, 1 self black : 1 tortoise : 1 self red, is quite close to expectation. Further proof of the correctness of this interpretation will be given in the forthcoming paper already referred to.

Although CASTLE (1912) described all the characters involved in the tricolor series of guinea-pigs and how one character might mask another, he made no attempt to enumerate the factors by which these char-

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[11] We have a fairly large amount of data on hand confirming this, but it is being reserved for a later paper.

acters are represented in the germ-cell. Neither did he attempt to explain the relation of the factors to each other. This has been attempted by LITTLE (1913, p. 42). The latter uses the symbol T to represent the self black condition. This corresponds to our E factor. The tortoise condition is represented by t, which corresponds to our e^b factor. In addition he postulates the factor E.

"$E = $ *extended black pigmentation necessary for the production of any* black hairs on the coat, and its allelomorph $e = $ the loss of black pigment from the coat, producing yellow animals.

"Thus, if a race of tortoise animals existed, formula *EEtt,* we should have a homozygous race of tortoise animals (which in fact exist). If, however, the tortoise animals were of the formula *Eett* we should expect young of three kinds:

(a) *EEtt* tortoise, (b) *Eett* tortoise, (c) *eett* yellow. Types (a) and (b) having E present would be able to form black pigment on the coat; but type (c), lacking E, would be *yellow* [red] in appearance, though it would be *potentially tortoise.*"[12]

If animals of the formula *eett* are self reds that are potential tortoises, then animals of the formula *eeTT* must be *self reds that are potentially self blacks.* Therefore, according to his interpretation, all self red animals are potentially either self blacks or tortoises. This leads to some difficulties. Suppose that a self red of the formula *eeTT* (and hence potentially a self black) were crossed with a tortoise, *EEtt,* all the offspring should be *EeTt,* or self blacks. We have crossed many self reds, extracted from self black matings, and which, therefore, according to LITTLE's hypothesis should carry the T factor, with tortoises, and have never obtained any self blacks.

If LITTLE's hypothesis is correct, heterozygous self black animals of the formula *EeTt* bred to self reds of the formula *eett* or *eeTt* should produce three kinds of offspring: self blacks, tortoises and self reds. We have mated a fairly large number of heterozygous self blacks, obtained in various ways, to self reds extracted from various kinds of matings and have always obtained either approximately half self blacks and half tortoises or half self blacks and half self reds, but never all three kinds of offspring from any one mating.

All the evidence we have at hand seems to indicate, therefore, that self reds are potentially neither self blacks nor tortoises, but simply what they appear to be, self reds. Such being the case, we feel justified in asserting that the relationship of self black, tortoise and self red cannot be adequately explained on the basis of two independent sets of allelomorphs.

[12] Original not italicized.

SUMMARY

1. The factors more or less directly concerned with tricolor inheritance in guinea-pigs are described and their inter-relations shown by means of table 1 and figure 1.

2. In the experimental results it is shown: (1) that tricolors may be of two kinds—those homozygous and those heterozygous for the e^p or black-spotting factor; (2) that black-and-whites of tricolor parentage are also either homozygous or heterozygous for the same factor; and (3) that red-and-whites of tricolor parentage may carry the e^p factor in a homozygous or heterozygous condition, or it may be altogether absent, in which event the red-and-white breeds true.

3. Red-and-whites may be tested for the presence of the black spotting factor by mating them to homozygous self reds. If the factor is present there will be tortoises among the offspring; if it is absent they will be all self reds.

4. Black-and-whites of the tricolor series, homozygous for e^p, produce only tortoises when mated to homozygous self reds; if they are $e^p e$, half of the offspring are tortoises and the other half self reds. Both these kinds of black-and-whites, therefore, produce *some* tortoises, but *no* self blacks when mated to self reds.

5. Black-and-whites carrying E may be of three kinds, (1) EE, (2) Ee^p and (3) Ee. The first kind when mated to homozygous self reds produce all self blacks; the second kind, half self blacks and half tortoises; the third kind, half self blacks and half self reds. Black-and-whites carrying E, therefore, *always* produce *some* self blacks when mated to self reds, but in some instances (when Ee^p) also produce tortoises.

6. EE and Ee black-and-whites were produced by mating self blacks to ce red-and-whites and inbreeding the F_1 self blacks. Some of the F_2 generation are black-and-whites which are either EE or Ee. The first kind when mated together breed true.

7. Animals of the tricolor series carrying a large amount of black pigmentation (e^p) and also a large amount of white spotting tend to produce a comparatively large number of black-and-whites and no e^p red-and-whites. Those carrying a small amount of black pigmentation tend to produce a comparatively large number of e^p red-and-whites and no black-and-whites.

8. The statement is made, but complete experimental proof is reserved for a later paper, that the three factors, E, complete extension

of black pigment, e^p, partial extension, and e, non-extension, form an allelomorphic series.

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