

AN EIGHT-FACTOR CROSS IN THE GUINEA PIG*¹

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THE STOCKS

An eight-factor cross in the guinea pig

The purpose of the present paper is to present data on the assortment, relative to each other and to sex, of eight pairs of Mendelian factors of the guinea pig. A stock, recessive in six color factors and in two factors which affect hair direction, was built up from various sources. This stock consisted of smooth furred, pink eyed, pale brown-yellow-white tricolors. A stock of self colored golden agoutis, smooth furred except for reversal of hair direction on the hind toes, was built up to supply the 8 dominant allelomorphs. The following are the series of factors involved.

S, s	s piebald (white areas) (incomplete dominance)
$E, e^p, (e) e^p$	tortoise shell (red areas), e self red
A, a	a black in place of agouti
$C, c^k, (c^d, c^r, c^a)$	c^k slight dilution of black, marked dilution of red
	c^d marked dilution of both black and red
	c^r slight dilution of black, white in place of red, red eyes
	c^a white in place of both black and red, pink eyes (albino)

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¹ A large portion of the data for this paper was obtained in experiments carried on by the author in the Bureau of Animal Industry, UNITED STATES DEPARTMENT OF AGRICULTURE.

<i>B, b</i>	<i>b</i>	brown in place of black, brown eyes, red unaffected
<i>P, p</i>	<i>p</i>	pale sepia in place of black, pink eyes, red unaffected
<i>R, r</i>	<i>R</i>	rough fur in place of smooth fur, at least on hind toes
<i>M, m</i>	<i>m</i>	extension of roughness to back and head (incomplete dominance of <i>M</i>)

DIFFICULTIES IN CLASSIFICATION

As might be expected, there were certain difficulties of classification among the 192 varieties expected in the backcross generation (*M, m* produce no visible effect except in the presence of *R*).

The piebald pattern (due primarily to *ss*) is subject to the action of modifiers which may cause the average percentage of white to vary from at least 10 to 90 percent. The average in the multiple recessive stock was 59 percent for males and 67 percent for females. There is also much variation which is not genetic and yet not due to environmental factors common to litter mates. Genetic piebalds (*ss*) occasionally show no white. It is not unlikely that homozygous self (*SS*) could be produced with a little white by selection for the appropriate minor factors. These cases were certainly so rare in the stocks used that there would have been no serious difficulty in classification into self and piebald were it not that dominance is incomplete. The heterozygotes are often completely self colored but rather more frequently they show a little white on nose or feet. Occasionally they range as high as 25 percent white in the coat, at least in females, with no more residual heredity for white than expected from the stocks used here. The distribution of percentages of white in the multiple recessive stock, in the F_1 matings of 3 homozygous dominant animals and in the backcross generation, are given in table 1. There is clearly some overlapping of the ranges of *Ss* and *ss* but separation of the males into "near self" and "piebald" at 7.5 percent white and of the females at 22.5 percent gives approximately equal numbers of the two classes and can involve no error that would seriously affect the recombination percentages.

The factor e^p (tortoise shell) was given preference over *e* (self red) in making up the multiple recessive stock, in order to avoid interference with the agouti series. Factor *e* was, however, carried in one of the foundation stocks, that used for introduction of *b*, and segregated out in a few backcross matings ($Ee \times e^p e$), in place of e^p . The distinction between the dominants (Ee^p) and the recessives ($e^p e^p$) from the typical backcross matings was ordinarily easy to make. There are rare cases (as noted by IBSEN 1919) in which animals which are undoubtedly $e^p e^p$ show no red or yellow spotting in the coat, and more frequent cases in which there are

TABLE 1
The amount of white in the coat in (A) the multiple recessive stock (ss), (B) among F₁'s from cross of multiple recessives with 3 multiple dominants proved to be homozygous (SS) by occurrence of no piebalds among 16, 24, and 26 young respectively, and (C) in the backcross generation excluding red-eyed dilutes.

MATING	SEX	AMOUNT OF WHITE IN COAT (parts in 20)																						
		0	x	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Multiple recessive stock (A) (ss × ss) 1924, 1925	♂	2	5	6	6	10	9	6	6	3	10	10	8	11	19	12	14	15	10	16	10	18	17	223
	♀	2	4	1	4	6	5	5	6	6	4	7	9	15	13	16	20	14	12	16	21	25	20	231
3 F ₁ Matings (SS × ss) (B)	♂	16	15	31
	♀	14	19	1	1	35
Backcross (Ss × ss) (C) (cc omitted)	♂	33	70	9	8	8	7	11	6	5	2	5	3	6	4	7	5	2	5	6	3	4	2	211
	♀	10	65	5	8	7	9	12	10	4	12	3	9	3	5	16	4	7	10	8	6	11	2	226

only a few hairs of these colors. The close approach to equality of recorded Ee^p and e^pe^p among dark-eyed young (115:113) indicates that there was little or no error among these but the deficiency of tortoise shells among the pink eyed ($134Ee^p:98e^pe^p$), although not certainly significant, may indicate some tendency to overlook yellow hairs in these animals in which black and brown are reduced to colors which do not contrast sharply with yellow. The crossover figures involving E are accordingly reported separately for dark-eyed and pink-eyed young. Another possible source of error in the separation of Ee^p from e^pe^p and also of Ss from ss came in the few cases in which factor c^r segregated out as the representative of the albino series. This factor reduces the red spotting of tortoise shells to white. Fortunately the pattern is typically so different from piebald that classification is usually possible. Among the 21 animals of formula c^rc^r , 2 were pink-eyed whites, resembling albinos, but undoubtedly of formula eec^rpp . These, of course, could not be classified with respect to piebald. Five had no white spotting of any sort and could thus only be called $SsEe^p$, 3 had a trace of white only in regions expected in heterozygous piebalds and were also called $SsEe^p$, 4 had white brindling characteristics of tortoise shells, with little or no piebald white, and were called Sse^pe^p , 4 had a large amount of white in typical piebald patterns only and were called $ssEe^p$, while 3 showed both typical piebald white and white brindling and were called sse^pe^p .

There was never any doubt about the classification into agouti (Aa) and non-agouti (aa), except in 12 animals which showed no dark pigment in the coat (ee , or e^pe^p with so much white that there happened to be no dark pigmented spot).

In the albino series, factor c^k was given preference over c^r and c^a as the recessive representative. Albinism (c^a) would have interfered with the expression of all of the other color factors and c^r raises the difficulty in the separation of piebald and tortoise shell noted above. Factor c^d , which dilutes black more obviously than does c^k , would have been preferable, but these factors were not distinguished until the somewhat long drawn out process of putting the recessive stock together had been nearly completed. It turned out then that c^d had been wholly lost from this stock which thus came to be largely c^kc^k but carried c^r sufficiently frequently to enable c^rc^r to appear in the back cross generation in the 21 animals referred to above (from matings $Cc^r \times c^kc^r$). There was no difficulty in distinguishing Cc^k from c^kc^k in cases in which there was red (Cc^k) or yellow (c^kc^k) in the fur, whether due to the agouti pattern (Aa) or to tortoise shell (e^pe^p). In animals of constitution Ee^paa , the grade of black or brown averages

somewhat lighter with $c^k c^k$ than with Cc^k but as previously shown (WRIGHT 1925, 1927) there is so much overlapping that classification is unsatisfactory. It has seemed best to omit all animals of this constitution in considering the relations of the C series.

There was no difficulty in distinguishing Bb and bb in animals which were not pink-eyed as both eye color and the difference between black and brown in the dark parts of the fur provide reliable criteria. Eye color fails in the pink-eyed varieties and the difference between pale sepia and pale brown is not always clear cut. It has seemed best to present the data for dark eyed (Pp) and pink eyed (pp) separately, although it is believed that there was no substantial amount of error in the latter.

There was never any difficulty in distinguishing Pp with black, brown or red eyes, from pp with pink eyes, albinism having been entirely absent from the recessive stock. A brown-pale brown-red-white quadricolor, mosaic in P and p , appeared in the backcross generation (WRIGHT and EATON 1926). This animal transmitted P in a mating with a pink-eyed animal and is here classified as Pp .

There was also no difficulty in distinguishing rough furred (Rr) from smooth (rr) (except in two animals of which only the heads were not eaten when found).

The rough furred fell easily into two approximately equal groups, full roughs, $Rrmm$, and partial roughs $RrMm$. The former have at least two pairs of dorsal rosettes, and rosettes about and between the eyes, while in the latter there is typically only one dorsal pair or even merely a dorsal crest and no head rosettes. A few doubtful, intermediate cases were assigned by drawing a somewhat arbitrary line. This factor M was derived from a different source from the modifier of full rough described in an earlier paper (symbol S , WRIGHT 1916). Nevertheless its behavior as followed in the production of the multiple dominant stock was exactly the same. Inasmuch as a similar modifier has been found homozygous in 3 wild species of cavy as well as in various domestic stocks of guinea pig there need be little hesitation in considering it as always the same. Smooths of formula $rrMM$ are to be considered as "type" from which the full rough $RRmm$ differs by 2 factors. In the production of the multiple recessive stock, all of the final stock (smooth) was derived by segregation from full rough parents ($Rrmm \times Rrmm$) in order to insure the absence of factor M .

ABSENCE OF SEX LINKAGE

The first crosses between the multiple dominant and recessive stocks were made before the former had been rendered completely homozygous.

There was in consequence segregation of one or more factors in most cases. In a typical case of a mating between a male dominant and a female recessive the young consisted of 11 partial rough golden agouti (4 ♂, 7 ♀) and 9 partial rough cream agouti (5 ♂, 4 ♀), indicating a mating of type $SSEEAA Cc^a BBPPRRMM \times sse^p e^p aac^k c^k bb p p r r m m$. The production of males dominant in all 8 factors in this and other similar matings proves that all 8 of the factors are transmissible from father to son and hence not sex linked, if the male is heterozygous for sex as in cats and man and as indicated by cytologic evidence for a number of other mammals. The cytologic situation is not certain in the case of the guinea pig. An X-Y pair is reported in the primary spermatocyte by STEVENS (1911) and by HARMON and ROOT (1926) but had not been identified in work reported by PAINTER (1926). However, it is clear from reciprocal crosses (female dominant by male recessive) that none of the 8 factors show sex linkage of the type in which the female is heterozygous for sex. A typical mating of this sort gave 8 partial rough agoutis (2 ♂, 6 ♀) and 4 partial rough agouti tortoise shells (all males) indicating segregation of E and e^p in the mother. Transmission of all 8 factors from mother to daughter was found in many other cases besides the 6 from this cross.

THE MONOHYBRID RATIOS

Fourteen F_1 males and 17 F_1 females, all partial rough agoutis with a little white in the fur in most cases, were tested by backcrossing to the recessive stock. The results for each pair of factors separately are given, by matings, in table 2. The males A, B and C and females a to i came from the cross of male dominant with female recessive, while the others came from the reciprocal cross.

There are two cases (matings F and T) in which it is clear from the results that the F_1 parent was not a heterozygous dominant with respect to piebald, but a recessive (ss). One of these (T) had 15 percent white in his coat and came from an F_1 mating which was proved to be $Ss \times ss$ by the production of well defined piebalds. The amount of white made it more probable than not that he would turn out to be a recessive in this respect, although another male (H) with 15 percent (as well as the male of G with 10 percent) turned out to be heterozygous. The rest of the males had only 5 percent or less white in the coat. The breeding record of mating F makes it certain nevertheless that this male (no white in coat!) also was genetically a piebald. The progeny of these matings are used in studying the recombination percentages except those involving S .

TABLE 2
Segregations with respect to each pair of allelomorphs separately from each F₁ male and F₁ female (some mated 2 or 3 times). Male of YZ was mated with Rr females and hence with expectation of 3R-:to1 rr instead of 1:1. Males of F and T proved to be ss. Females of u, v, and w proved to be AABBPP.

	NUMBER	♂	♀	S	s	E	e ⁷ e	A	a	C	c ^r	B	b	P	p	R	r	M	m
A, G	30	10	20	14	16	18	12	13	17	4	14	20	10	9	21	12	18	5	7
B	9	6	3	6	3	3	6	5	4	3	4	4	5	4	5	3	6	0	3
F	13	9	4	(0	13)	6	7	7	5	7	4	7	6	6	7	8	5	3	5
H	12	7	5	4	6	3	9	2	4	6	4	5	4	6	6	6	6	1	5
I	28	13	14	16	11	14	13	11	17	11	9	17	11	14	14	14	14	7	7
J, Q, R	40	23	17	24	16	21	19	14	25	14	10	21	18	23	17	21	19	7	14
K	14	5	9	8	6	7	7	6	8	3	7	8	6	4	10	7	7	5	2
L, M	29	16	13	14	15	18	11	20	9	17	7	15	14	20	9	16	13	5	11
N	3	0	3	2	1	2	1	2	1	2	0	1	2	2	1	1	2	0	1
O	7	2	5	1	6	2	5	3	3	2	4	3	3	4	3	6	1	5	1
S, V	34	14	20	18	15	21	12	12	22	12	9	19	14	20	14	12	22	9	3
T	14	6	8	(0	14)	7	7	7	6	6	7	7	6	4	10	11	3	8	3
W, X	17	4	13	9	8	10	7	6	11	4	5	7	10	8	9	9	8	5	4
Y, Z	19	9	10	10	9	7	12	10	8	8	8	9	9	8	11	(12	7)	6	6
Total (1:1)	269	124	144	126	112	139	128	118	140	99	92	143	118	132	137	126	124	66	72
Dev. ± SE		10.0 ± 8.2		7.0 ± 7.7		5.5 ± 8.2		11.0 ± 8.0		3.5 ± 6.9		12.5 ± 8.1		2.5 ± 8.2		1.0 ± 7.9		3.0 ± 5.9	
Prob.		.22		.36		.50		.17		.61		.12		.76		.90		.61	
(0:1) & (3:1) unclassified		1		(0	4	2		11		78		8				(12	7)		

a	34	17	17	16	18	17	17	11	18	16	18	16	17	17	8	9
b	6	4	2	4	3	3	3	2	1	5	5	1	4	2	2	2
c	24	16	8	13	16	8	16	9	13	11	9	15	12	12	8	4
d	2	1	1	1	1	2	0	0	1	1	2	0	1	1	1	0
e	3	1	2	0	3	1	2	1	1	2	0	3	0	3	0	0
f	3	2	1	1	3	0	2	1	1	2	2	1	2	1	1	1
h	4	1	3	0	2	2	2	0	2	2	2	2	3	1	1	2
i	7	6	1	3	6	1	2	5	2	5	3	4	4	3	3	1
j	5	3	2	3	1	4	3	2	2	3	2	3	2	3	2	1
k	17	8	8	9	13	4	10	7	13	4	10	7	8	9	3	5
l,r	15	5	10	7	10	5	9	6	7	8	4	11	7	8	6	1
m	16	7	8	9	8	8	10	6	7	9	8	8	4	11	4	0
n,s	26	11	14	15	14	11	16	9	13	12	16	10	18	7	6	12
o	21	8	13	10	9	12	7	14	8	13	9	12	8	13	1	7
p	3	3	0	1	2	1	1	2	2	1	2	1	3	0	1	2
t	8	2	6	5	5	3	3	5	2	6	3	5	4	4	2	2
u,v,w	27	14	13	12	15	17	(26	0)	(27	0)	(27	0)	9	18	5	4
Total (1:1)	221	109	109	105	115	120	106	87	91	79	93	100	107	112	54	53
Dev. ± SE		0.0 ± 7.4		5.0 ± 7.4		10.0 ± 7.4	9.5 ± 6.9		6.0 ± 6.5		3.5 ± 6.9		1.0 ± 7.0		2.5 ± 7.4	0.5 ± 5.2
Prob.		1.00		.50		.18	.17		.36		.61		.88		.74	.92
(1:0)		3		1		1	(26	2		51	(27	0)	(27	0)	2	
unclassified																
(1:1) ♂ + ♀ tests	490	233	253	231	227	259	224	227	190	171	236	218	228	235	323	236
Dev. SE		10.0 ± 11.0		2.0 ± 10.7		15.5 ± 11.0	1.5 ± 10.6		9.5 ± 9.5		9.0 ± 10.7		3.5 ± 10.8		1.5 ± 10.8	2.5 ± 7.8
Prob.		.36		.85		.16	.89		.32		.40		.75		.89	.75

TABLE 3
Progeny from backcross test of F₁ males. The matings are indicated by letters as in table 2 and the litter by a subscript. Matings F, T, Y and Z are put in parentheses. F and T come from F₁ males which proved to be genetically piebald (ss) and the male of Y and Z was mated with heterozygous rough females. The symbols have the following meanings: B—Black; pBr—pale Brown; W—White; (Br)—Brown eye; Br—Brown eye; Br—Brown eye; Br—Red; Ag—Agouti; (P)—Pink eye; pS—pale Sepia; Y—Yellow (or cream); (B)—Black eye; Sm—Smooth; RfA—Full Rough; RfC—Partial Rough. Such a combination as BrYAg-Y(Br) means brown yellow agouti with yellow spotting and (brown eye).

	NEAR SELF				PIEBALD							
	Full Rough		Partial Rough		Smooth		Full Rough		Partial Rough		Smooth	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
BRAG (B) BYAG (B) BrRAG (Br) BrYAG (Br)	J ₁	L ₆ I ₉ * A ₃ K ₄	R ₃ L ₁₂	V ₃ I ₁₀	I ₇ L ₁₀	J ₃ K ₄ L ₃ L ₁₂ I ₂ *	M ₂ (F ₅)	Q ₃	(Z ₂)	V ₃ H ₂ L ₄	L ₆ (Y ₁) V ₄ L ₆	A ₃ V ₃
B (B) Br (Br)	V ₉	A ₁₀	J ₃ L ₁	R ₇ S ₁ * V ₉ J ₄	Q ₂ X ₂ B ₁ K ₇ L ₂ R ₇ V ₂	N ₁ Q ₃	(F ₂) J ₃	X ₃ I ₄	A ₁₀ M ₂	A ₉ V ₃ O ₂	(F ₁)I ₆ J ₄ L ₉ R ₃ V ₃	I ₁ *Q ₃ V ₃ K ₇ W ₂
BRAG-R (B) BYAG-Y (B) BrRAG-R (Br) BrYAG-Y (Br)	B ₃ (Y ₂)	A ₄ S ₁ Z ₄		O ₁	B ₃ I ₄ G ₁ J ₄	N ₁ H ₃ I ₃ * L ₁₁ X ₁ B ₂ R ₄	L ₂ R ₂	R ₃ (Z ₁)		A ₇ (T ₃)	L ₇ A ₇ I ₆ R ₃	O ₁ L ₁ W ₁
B-R (B) B-Y (B) Br-R (Br) Br-Y (Br)	I ₃ R ₃ M ₁ J ₃	I ₅	X ₃ V ₅	L ₉ W ₅ (Y ₂) J ₃	Q ₂ I ₆ V ₅ V ₆	W ₆ V ₇ (Z ₃)	H ₃	(F ₂) (F ₃)	V ₁ I ₆ O ₂	(Y ₃) (T ₂) (T ₃) ₄		V ₄ V ₃ L ₇ (T ₂)

One F_1 female (mating r) produced no non-agoutis (aa) no browns (aa) and no pink-eyed (pp) among 27 young and was clearly $SsEe^pAACc^k BBPPRrMm$. There was no doubt about her identity because of a certain peculiarity in her rosette pattern recorded at birth and there was nothing in the records to indicate that her dam (a multiple dominant) had ever mated with any other male than her reputed sire (a multiple recessive). Whatever may be the explanation, it has seemed safe to include her progeny in the recombination data except where A , B and P are concerned.

One of the F_1 males was mated with two females which were full roughs (heterozygous) although recessive in the seven other respects (matings Y and Z). The progeny are used in estimating all crossover percentages except those involving R .

Examination of the totals in table 2 shows that the monohybrid ratios are all reasonably close to 1:1 although as noted above it is not unlikely that a few genetic tortoise shells are classed as self. The chance of obtaining a more divergent system of values for the 9 cases (including sex) is .85 by the χ^2 method (9 degrees of freedom). It must be remembered, however, that in the case of S,s and M,m , the overlapping classes were separated somewhat arbitrarily.

THE DIHYBRID RATIOS

The results of the backcross generation from the F_1 males are given in detail in table 3, the mating and litter of each animal being indicated by the entries. Table 4 deals similarly with the progeny of the F_1 females. All of the data are included either in the body of the table or below. The cases which cannot be used for every crossover test are indicated as described in the titles.

The combinations suitable for determining possible linkage relations between each pair of factors are abstracted in tables 5 and 6 together with the calculations of the crossover percentages and the chances of exceeding each by random sampling. Inspection of these reveals not one, either in the male or female tests, which can be said to differ significantly from 50 percent. The most probable cases of linkage among the male tests are those of AM and BR with indicated crossover percentages of 43.4 and 45.3 percent respectively. The chance of exceeding by random sampling are .08 and .14 in the two cases. The reality of these cases is not confirmed by the female test. The strongest indication among the latter is that of C and B , 45.8 percent with a probability of being exceeded by chance of about 1 in 3 and no confirmed by the close approach to 50 percent (49.2 percent) among the males. It will be seen that the deviations

TABLE 5

The combination of each two sets of allelomorphs in backcross tests of F_1 males. The two original combinations are indicated in the column headings by XY and xy (double dominant and double recessive respectively) while the cross combinations are indicated by Xy and xY following in the order of the factors in the first column. The backcross ratio (1:1:1:1) is disturbed in the case of EC and AC by omission of Ea in which the separation of C and c^b was not satisfactory and in the case of RM by omission of smooths in which M and m produce no visible effect at all. The last column gives the probability of obtaining at least as great a deviation from 50% in either direction by random sampling. The value of χ^2 from the numbers of crossovers and linkages is 20.0 indicating a probability of .86 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NO.	CROSSOVERS		PROBABILITY
						No.	Percent	
SE	64	62	62	50	238	124	52.1	.52
SA	56	68	47	60	231	115	49.8	.95
SC	49	38	37	40	164	75	45.7	.27
SB	70	54	58	52	234	112	47.9	.51
SP	68	58	54	58	238	112	47.1	.36
SR	51	65	55	48	219	120	54.8	.16
SM	26	31	28	33	118	59	50.0	1.00
EA	62	77	55	62	256	132	51.6	.62
EC	32	30	67	61	190	97	51.1	.77
EB	73	66	69	52	260	135	51.9	.54
EP	66	73	66	62	267	139	52.1	.50
ER	70	62	55	61	248	117	47.2	.37
EM	35	40	30	32	137	70	51.1	.80
AC	57	61	37	25	180	98	54.4	.23
AB	68	49	73	67	257	122	47.5	.42
AP	59	59	69	71	258	128	49.6	.90
AR	54	54	68	64	240	122	50.8	.80
AM	25	36	41	32	134	77	57.5	.08
CB	55	42	48	38	183	90	49.2	.82
CP	53	46	42	50	191	88	46.1	.28
CR	52	39	37	47	175	76	43.4	.08
CM	26	32	20	21	99	52	52.5	.61
BP	72	71	60	58	261	131	50.2	.95
BR	74	60	50	59	243	110	45.3	.14
BM	40	39	26	31	136	65	47.8	.61
PR	62	62	64	62	250	126	50.4	.90
PM	35	33	31	39	138	64	46.4	.39
RM	60	66	126	66	52.4	.59

TABLE 6

The combination of each two sets of allelomorphs in the backcross tests of F_1 females. Headings as in table 5. The value of χ^2 from the numbers of crossovers and linkages is 13.2 indicating a probability of .992 of obtaining at least as great deviations by random sampling (28 degrees of freedom).

	XY	Xy	xY	xy	TOTAL NUMBER	CROSSEOVERS		PROBABILITY
						Number	Percent	
SE	60	44	60	55	219	104	47.5	.46
SA	49	44	56	43	192	100	52.1	.56
SC	45	35	45	44	169	80	47.3	.49
SB	42	50	50	50	192	100	52.1	.56
SP	48	45	48	52	193	93	48.2	.61
SR	51	53	56	59	219	109	49.8	.94
SM	25	26	29	27	107	55	51.4	.77
EA	59	51	46	36	192	97	50.5	.89
EC	37	32	53	47	169	85	50.3	.94
EB	54	56	39	44	193	95	49.2	.83
EP	49	61	47	36	193	108	56.0	.10
ER	60	60	47	52	219	107	48.9	.74
EM	33	27	21	26	107	48	44.9	.29
AC	56	50	19	17	142	69	48.6	.74
AB	52	53	41	46	192	94	48.9	.77
AP	53	53	42	45	193	95	49.2	.83
AR	52	52	46	41	191	98	51.3	.72
AM	27	25	22	24	98	47	48.0	.69
CB	40	35	30	37	142	65	45.8	.31
CP	35	41	40	27	143	81	56.6	.11
CR	39	50	40	49	168	90	53.6	.35
CM	21	18	18	22	79	36	45.6	.43
BP	45	48	51	49	193	99	51.3	.72
BR	45	47	53	47	192	100	52.1	.56
BM	20	25	29	24	98	54	55.1	.31
PR	49	47	49	47	192	96	50.0	1.00
PM	24	25	25	24	98	50	51.0	.84
RM	54	53	107	53	49.5	.92

from 50 percent are even smaller on the whole than might be expected by chance.

As already noted, the classification of factors *E* and *B* was easy in dark-eyed animals but was more difficult and accompanied occasionally by a feeling of uncertainty in the pink-eyed ones. It seems desirable therefore to examine the crossover percentages of these groups separately. The

TABLE 7

The crossover percentages from tests of F_1 males in the cases involving E and B , divided according to the presence of Pp or pp . In the former case, separation of Ee^p from E^pe^p and Bb from bb was easy and wholly reliable. In the latter cases there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (Pp)				PINK EYED YOUNG (pp)			
	Total Number	Crossovers		Prob.	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	122	65	53.3	.47	116	59	50.9	.85
SB	122	62	50.8	.86	112	50	44.6	.25
EA	128	67	52.3	.60	128	65	50.8	.86
EC	95	52	54.7	.36	95	45	47.4	.61
EB	132	58	43.9	.16	128	77	60.2	.02
EP	132	66	50.0	1.00	135	73	54.1	.34
ER	124	62	50.0	1.00	124	55	44.4	.21
EM	68	30	44.1	.33	69	40	58.0	.19
AB	128	55	43.0	.11	129	67	51.9	.66
CB	95	50	52.6	.61	88	40	45.5	.39
BP	132	60	45.5	.30	129	71	55.0	.25
BR	124	55	44.4	.21	119	55	46.2	.41
BM	68	32	47.1	.63	68	33	48.5	.81

TABLE 8

The crossover percentages from tests of F_1 females in cases involving E and B , divided according to the presence of Pp or pp . In the former case separation of Ee^p from e^pe^p and Bb from bb was easy and wholly reliable. In the latter case there was more difficulty and occasionally uncertainty.

	BLACK OR BROWN EYED YOUNG (Pp)				PINK EYED YOUNG (pp)			
	Total Number	Crossovers		Probability	Total Number	Crossovers		Probability
		Number	Percent			Number	Percent	
SE	123	57	46.3	.42	96	47	49.0	.84
SB	96	51	53.1	.54	96	49	51.0	.84
EA	95	46	48.4	.76	97	51	52.6	.61
EC	102	50	49.0	.84	67	35	52.2	.71
EB	96	48	50.0	1.00	97	47	48.5	.76
EP	96	47	49.0	.84	97	61	62.9	.01
ER	123	59	48.0	.65	96	48	50.0	1.00
EM	58	24	41.4	.19	49	24	49.0	.89
AB	95	50	52.6	.61	97	44	45.4	.36
CB	75	37	49.3	.91	67	28	41.8	.18
BP	96	51	53.1	.54	97	48	49.5	.92
BR	96	46	47.9	.68	96	54	56.3	.22
BM	49	26	53.1	.67	49	28	57.1	.32

necessary data are given in tables 7 and 8. The crossover percentages are somewhat more variable, as expected with the smaller numbers, but none differ significantly from the expectation under random sampling either in the dark-eyed or pink-eyed, except perhaps the figure of 62.9 percent for *E* and *P* in pink-eyed young in the female test and that of 60.2 percent

TABLE 9

The crossover percentages from tests of F₁ males and F₁ females combined. The value of χ^2 from the numbers of crossovers and linkages is 12.25 indicating a probability of .995 of obtaining at least as great a series of deviations from equality by random sampling (28 degrees of freedom).

	TOTAL NUMBERS	CROSSOVERS		PROBABILITY
		Number	Percent	
SE	457	228	49.9	.96
SA	423	215	50.8	.73
SC	333	155	46.5	.21
SB	426	212	49.8	.92
SP	431	205	47.6	.31
SR	438	229	52.3	.34
SM	225	114	50.7	.84
EA	448	229	51.1	.64
EC	359	182	50.7	.79
EB	453	230	50.8	.74
EP	460	247	53.7	.11
ER	467	224	48.0	.38
EM	244	118	48.4	.61
AC	322	167	51.9	.50
AB	449	216	48.1	.42
AP	451	223	49.4	.81
AR	431	220	51.0	.66
AM	232	124	53.4	.29
CB	325	155	47.7	.41
CP	334	169	50.6	.83
CR	343	166	48.4	.55
CM	178	88	49.4	.88
BP	454	230	50.7	.78
BR	435	210	48.3	.47
BM	234	119	50.9	.79
PR	442	222	50.2	.92
PM	236	114	48.3	.60
RM	233	119	51.1	.74

for *E* and *B* in the pink-eyed young in the male test. The former figure may readily be explained by the failure to recognize a small yellow patch in a few pink-eyed tortoise shells.

As only slight differences have been found in the crossover percentages of males and females in mammals (rats, mice and rabbits) it is probably legitimate to combine the male and female tests. The results are given in table 9. Here again there is no case which differs significantly from 50 percent, the extremes being 46.5 percent and 53.7 percent. The probability of obtaining a system of at least as great deviations (χ^2 test) by random sampling is the embarrassingly high figure of .995. The grand average recombination percentage for the 28 comparisons is 49.9 percent. It appears that for the present these 8 factors and sex must be considered as isolated representatives of 9 different linkage systems. There remain, of course, the possibility that some factors may be found showing appreciable linkage with two of the above and thus binding them into the same system.

THE OCTOHYBRID RATIO

Before leaving the data there are two other aspects from which it will be desirable to examine the randomness of assortment. First consider the combinations with respect to all 8 factors instead of merely by 2's.

The data are condensed in table 10 by disregarding the sex of the F_1 parent and of the offspring. Matings F, T, Y, Z and v are omitted because of the imperfections discussed above and mating D because of the production of 6 self reds in which classification by the agouti series could not be made. The few other imperfect individual records are also omitted. The 398 young left include 143 of the 168 distinguishable varieties expected in the backcross generation. Among the 96 classes in which expectation is $1/256$ (or 1.56 in 398) there is variation from 0 to 4 individuals. Among the 56 classes with expectation $2/256$ (or 3.11 in 398) there is variation from 0 to 8. The 8 classes with expectation of $4/256$ (6.22 in 398) vary from 4 to 11 individuals. As these variations may appear greater than probable, it is worth while testing them against the distribution expected under random sampling which is approximately that of the Poisson series for mean values of 1.56, 3.11 and 6.22 respectively. Table 11 shows the comparison between observation and expectation on this basis. The χ^2 test, combining adjacent groups so as to leave none smaller than 4, indicates a probability of getting at least as great a system of divergences by random sampling of .33. Clearly there are no important departures from a random distribution.

TABLE 10

Segregation in backcross generation, combining tests of F_1 males and F_1 females, ignoring sex and omitting records of two males (F, T) found to be *ss*, of one male (H) found to be *Ee* instead of *Ee^p* and producing 6 *ee* young unclassifiable with respect to A and a, and one female (of *u, v, w*) proved to be *AABBPP*, and one male (YZ) which was mated with females heterozygous for Rough (*Rr*). All of the young from the other matings are included except 7 which were unclassifiable in one or more respects.

CHARACTERS	GENES	1 NEAR SELF			PIEBALD		
		Rf AB	Rf CD	Smooth	Rf AB	Rf CD	Smooth
		SRm	SRM	Sr-	SRm	srM	sr-
BRAg (B)	<i>EACBP</i>	1	2	6	3	1	1
BYAg (B)	<i>C^kB</i>	3	3	4	0	2	5
BrRAg (Br)	<i>Cb</i>	2	2	0	0	4	3
BrYAg (Br)	<i>c^kb</i>	3	0	3	0	2	3
B (B)	<i>Ea-BP</i>	3	5	4	3	4	9
Br (Br)	<i>-b</i>	2	5	11	2	2	6
BrRAg-R (B)	<i>e^pACBP</i>	2	1	1	3	0	5
BYAg-Y (B)	<i>c^kB</i>	1	2	3	2	2	3
BrRAg-R (Br)	<i>Cb</i>	2	0	4	1	1	2
BrYAg-Y (Br)	<i>c^kb</i>	1	0	8	3	1	3
B-R (B)	<i>e^paCBP</i>	3	4	5	0	0	1
B-Y (B)	<i>c^kB</i>	1	0	1	2	3	0
Br-R (Br)	<i>Cb</i>	3	0	6	1	2	2
Br-Y (Br)	<i>c^kb</i>	2	2	1	0	2	3
pSRAg (P)	<i>EACBp</i>	3	2	4	2	0	4
pSYAg (P)	<i>c^kB</i>	1	1	4	2	3	1
pBrRAg (P)	<i>Cb</i>	2	1	3	3	2	2
pBrYAg (P)	<i>c^kb</i>	1	0	2	2	0	8
pS (P)	<i>Ea-Bp</i>	3	3	6	8	1	8
pBr (P)	<i>-b</i>	3	6	9	4	6	7
pSRAg-R (P)	<i>e^pACBp</i>	1	1	3	2	3	3
pSYAg-Y (P)	<i>c^kB</i>	1	1	3	1	0	2
pBrRAg-R (P)	<i>Cb</i>	2	2	1	0	2	3
pBrYAg-Y (P)	<i>c^kb</i>	2	0	3	1	1	1
pS-R (P)	<i>e^paCBp</i>	1	1	6	2	1	4
oS-Y (P)	<i>c^kB</i>	1	1	3	2	2	2
pBr-R (P)	<i>Cb</i>	1	0	2	0	1	0
pBr-Y (P)	<i>c^kb</i>	1	0	1	1	3	3

COMPARISON OF LITTERMATES

It is next desirable to see whether the data show any tendency toward resemblance of littermates in factorial constitution. In particular it is

TABLE 11

Distribution of class frequencies in Table 10 under each mean Mendelian expectation (1/156, 2/256, or 4/256) compared with random distribution as calculated from Poisson series with means of 1.56, 3.11 and 6.22 respectively. χ^2 for 12 degrees of freedom is 11.44 indicating a probability of 0.33 of obtaining at least as great deviations from theory by random sampling.

NUMBER IN CLASS	CLASSES WITH MEAN MENDELIAN EXPECTATION OF								
	1/256 1.56 in 398			2/256 3.11 in 398			4/256 6.22 in 398		
	O	C	O-C	O	C	O-C	O	C	O-C
0	22	20.3	+1.7	3	{ 2.9		{ 0	0.0	
1	29	31.5	-2.5	10	{ 8.9	+1.2	{ 0	0.1	
2	29	24.5	+4.5	10	13.8	-3.8	{ 0	0.3	
3	14	12.7	+1.3	20	14.3	+5.7	{ 0	0.6	
4	2	{ 4.9	-5.0	8	11.1	-3.1	{ 1	1.0	
5	0	{ 1.5		5	6.9	-1.9	{ 0	1.2	
6	0	{ 0.4		5	{ 3.6	+1.9	{ 2	1.3	-1.5
7	0	{ 0.2		0	1.6		{ 1	1.1	+1.5
8	0			3	{ 0.6		{ 1	0.9	
9	0			0	{ 0.2		{ 2	0.6	
10	0			0	{ 0.1		{ 0	0.4	
11	0			0			{ 1	0.5	
Total	96	96.0		64	64.0		8	8.0	
Individuals	137			201			60		

important to determine whether the number of littermates which resemble each other in all factors, including sex, is so great as to indicate the frequent occurrence of uniovular twins. Table 12 shows the number of agreements between littermates classified as $\sigma^1 \sigma^1$, $\sigma^1 \varphi$ and $\varphi \varphi$ with respect to the S, E, A, B, P and R series. The M series is omitted because the possibility of classification of two littermates in this respect requires that both be rough furred and the C series is omitted for similar reasons. Matings F, T, Y, Z, and U are, of course, omitted. The symmetry of the results about 3 agreements, 3 disagreements in each case indicate that agreement is largely by chance. This has been tested by combining all into one table treating sex as a 7th factor and comparing the distribution with the expansion of $(\frac{1}{2}A + \frac{1}{2}D)^7$ where A and D are agreements and disagreements respectively. The close approach of the observed data to the theoretical distribution is obvious and is confirmed by a probability from χ^2 of .90. There is thus no tendency for littermates to resemble each other more than animals picked at random.

TABLE 12

Distribution of agreements and differences between littermates in sex and the six series S, E, A, B, P and R. Comparison is made with expectation from random sampling based on the expansion of $(\frac{1}{2}A + \frac{1}{2}D)$.⁷ Matings F, T, Y, Z and u, v, w, are omitted because of inequality of chances of agreement and difference in one or more of the above respects. The probability of obtaining at least as great deviations from theory by random sampling is 0.90 from $\chi^2 = 1.65$ for 5 degrees of freedom.

AGREE	DIFFER	♂ ♂	♂ ♀	♀ ♀	TOTAL	Calculated	DIFFERENCE
0	7	..	3	..	3	3.1	-0.1
1	6	2	17	0	19	21.3	-3.2
2	5	9	49	11	69	64.0	+5.0
3	4	25	50	25	100	106.6	-6.6
4	3	32	45	28	105	106.6	-1.6
5	2	25	16	25	66	64.0	+2.0
6	1	6	3	17	26	21.3	+4.7
7	0	2	..	0	2	3.1	-1.1
		101	183	106	390	390.0	0.0

There were, however, two cases in which there was agreement in sex and in the 6 factors. There was indeed an 8th agreement in each case. Two male littermates, both brown with a trace of white were both partial roughs and thus both ♂ *SsEe^aaabbPpRrMm* with uncertainty only in the albino series. Two other male littermates were both smooth pink-eyed pale sepias, one with no white, the other with a trace. While differing distinctly in grade of sepia, both were so pale as to be probably *c^kc^r* (both ♂ *SsEe^aaac^kc^rBBpprr*). These two cases may have been uniovular twins but as 3 cases of agreement in all of the respects dealt with in table 12 are expected by chance, little confidence can be placed in this. It is certain that such twins are so rare as to be of no importance in disturbing the randomness of Mendelian results.

DISCUSSION

The evidence for random assortment of *S, E, A, C, B, P, R, M* and sex, presented here is in harmony with the rather scanty data hitherto published. CASTLE (1916) found a close approach to random assortment in 108 F₂ animals from a cross of golden agouti (wild *Cavia cutleri*) (*EEAACCB*) and albino guinea pig, *eeaac^cbb*. There was also random assortment in 116 F₂'s from a cross of *Cavia Cutleri* with brown-eyed creams, *eeaac^dbb* as far as *E, A,* and *B* were concerned, classification by intensity (*C, c^d*) not having been attempted. The present writer (1916) found $32/75 = 43$ percent recombination between *R* and *M*, $34/53 = 64$

percent between *R* and *A* and $35/64 = 55$ percent between *A* and *M*, and found recombination to occur between *R* and *E*, *B*, *C* and *P* and between *M* and *B*, *C*, *P* and in a later paper (1923) noted the completely random assortment *S* and *E* in 11 successive backcrosses to an inbred tricolor stock. IBSEN (1923) reported the following ratios of non-crossovers to crossovers:

	<i>N</i> *	<i>X</i> *		<i>N</i> *	<i>X</i> *		<i>N</i> *	<i>X</i> *
EA	5	10	AC	7	4	CP	65	67
EC	43	55	AB	9	4	CR	22	27
EB	19	9	AP	7	9	BP	10	18
EP	82	73	AR	9	5	BR	10	11
ER	29	31	CB	11	6	PR	47	28

**n* = noncrossover. *x* = crossover.

IBSEN and GREGORY (1924) report independent assortment of *P* and a factor *Sm* (salmon eye) not belonging to any of the series discussed here and the present writer (1927) has found no linkage between another color factor, *F* and factors *C* and *P* among 242 F_2 's, which data incidentally gave further evidence of the complete independence of factors *C* and *P* of each other.

The most interesting cases are perhaps this case of *C* and *P* and that of *C* and *B*. Factors with effects similar to those of *C* and *P* in the guinea pig have been found to be linked in both the mouse and rat with crossover percentages between 10 and 20 percent in both cases (CASTLE 1919). The albino series (*C*) is linked with the brown series (*B*) in the rabbit (41 percent crossover) (CASTLE 1924). The apparent absence of linkage in these cases in the guinea pig does not necessarily prove that the factors are not homologous in the different rodents as there may well have been radical chromosome reorganizations since their remote origin from common ancestry, a phenomenon indicated by probable differences in chromosome number.

The chromosome number in guinea pigs, however, seems to be far from certain. Estimates published in the past 20 years range from 8 for the haploid number (LAMS 1913) to 30 (PAINTER 1926). STEVENS (1911) found 28 and HARMON and ROOT (1926) found 19. The independence of the first 9 factors tested in the guinea pig (including sex) is difficult to reconcile, with a haploid count of 8 but is not at all surprising if one of the larger numbers proves correct.

SUMMARY

Production of two stocks of guinea pigs carrying 8 dominant and 8 recessive factors, respectively, made possible a simultaneous test for possible linkage relations between each two of the factor series designated by *S*, *E*, *A*, *C*, *B*, *P*, *R*, and *M*.

A backcross generation of 269 individuals from the (largely) octohybrid males showed completely random assortment and similarly with a backcross generation of 221 from (largely) octohybrid females. None of the factors showed sex linkage either of the XY type or the ZW type.

143 of the 168 visibly distinct combinations expected in the backcross generation were obtained among 398 young from completely octohybrid backcrosses. It is shown that the distribution of the combinations, considering all factors at once, is random.

There is no indication of a tendency for littermates to resemble each other more than expected by chance. Two possible cases of uniovular twins were found where three cases of resemblance in all non-interfering respects was expected by chance.

The independence of the first 9 factors tested is not surprising if the large number of chromosomes reported for the guinea pig recently is correct.

LITERATURE CITED

- CASTLE, W. E., 1916 An expedition to the home of the guinea pig and some breeding experiments with material there obtained. Carnegie Inst. of Washington, Publ. No. 241, pp. 1-55.
- 1919 Studies of heredity in rabbits, rats and mice. Carnegie Inst. of Washington, Publ. No. 288.
- 1924 On the occurrence in rabbits of linkage in inheritance between albinism and brown pigmentation. Proc. Nat. Acad. Sci. **10**: 486-488.
- HARMON, MARY T., and ROOT, F. P., 1926 Number and behavior of the chromosomes of *Cavia cobaya* (the common guinea pig). Biol. Bull. **51**: 73-84.
- IBSEN, H. L., 1919 Tricolor inheritance. IV. The triple allelomorphous series in guinea pigs. Genetics **4**: 597-606.
- 1923 Evidence of the independent inheritance of 6 pairs of allelomorphs in guinea pigs. Anat. Rec. **23**: 392.
- IBSEN, H. L., and GREGORY, P. W., 1924 The salmon eye gene in guinea pigs. Anat. Rec. **25**: 140.
- LAMS, H., 1913 Etude de l'oeuf de *Cobaya* aux premiers stades de l'embryogenese. Arch. de Biol. **28**: 228-322.
- PAINTER, T. S., 1926 The chromosomes of rodents. Science N. S. **64**: 336.
- STEVENS, N. M., 1911 Heterochromosomes in the guinea pig. Biol. Bull. **21**: 155-167.
- WRIGHT, S., 1916 An intensive study of the inheritance of color and of other characters in guinea pigs with especial reference to graded variations. Carnegie Inst. of Washington Publ. 241, pp. 59-121.

- 1923 The relation between the piebald and tortoise shell color patterns in guinea pigs. *Anat. Rec.* **23**: 393.
- 1925 The factors of the albino series of guinea pigs and their effects on black and yellow pigmentation. *Genetics* **10**: 223-260.
- 1927 The effects in combination of the color factors of the guinea pig. *Genetics* **12**: 530-569.
- WRIGHT, S., and EATON, O. N., 1926 Mutational mosaic coat patterns of the guinea pig. *Genetics* **11**: 333-351.