

AN ANALYSIS OF VARIABILITY IN NUMBER OF DIGITS IN AN INBRED STRAIN OF GUINEA PIGS*

SEWALL WRIGHT

University of Chicago, Chicago, Illinois

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THE DIGITS OF GUINEA PIGS

Guinea pigs normally have four digits on the front feet (no thumb) and three digits on the hind feet (digits I and V absent). This condition is found in all wild species of the family Caviidae and in the closely allied family Hydrochoeridae.

A larger number of digits is found in general in the species of other families of the hystricoid rodents. The reduction in the Caviidae and Hydrochoeridae is thus in all probability a very ancient character.

It is a frequently stated principle of paleontology (DOLLO'S law) that lost parts never return. Nevertheless, it is well known that an extra digit, resembling in every respect (when well-developed) a normal little toe is not an especially uncommon occurrence in the guinea pig. CASTLE (1906) found a guinea pig with one imperfectly developed little toe and was able by selection and inbreeding to build up a race in which this digit was invariably present and perfectly developed including bones, muscles and nail and even a new plantar tubercle on the foot. I have found a similar little toe in several independent stocks. It has also been described by STOCKARD (1930) and by PICTET (1932). The appearance of the little toe in two grades of development is shown in figure 1. Its position and structure have been the same in all stocks in which it has been observed. This type of polydactyly must be sharply distinguished from the duplication of a digit which has occurred associated with other abnormalities of the foot (figure 2) in a few cases in stocks which have no special tendency toward the development of the reversionary type.

A series of grades of development of the little toes has been used in the records but for the purposes of this paper two will suffice: "Good" in which both little toes are full-sized and so firm that they do not bend back laterally to the foot on moderate pressure, and "poor" including all lower grades. Grades of perfection are recognizable within the category "good" but the above line of cleavage seems to be the most objective one which can be made.

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PREVIOUS GENETIC RESULTS

CASTLE (1906) found that the extra-toed condition was transmissible both by males and females and that the degree of transmission was closely related to the grade of development. His original male with only a weak fourth toe on one foot had 25 percent (9 out of 36) extra-toed young from

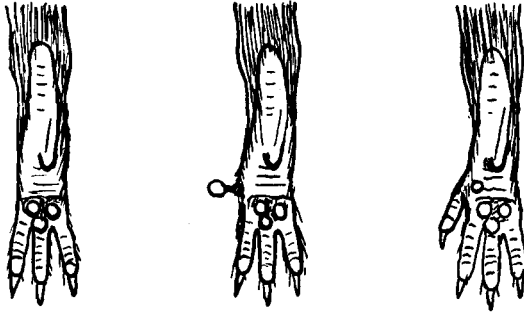


FIGURE 1.—Sketches of left hind feet of guinea pigs. The normal 3-toed condition is shown at the left. At the right is a foot with well-developed little toe and a corresponding plantar tubercle. Between is a grade of imperfect development of the little toe.

related females and 6 percent (2 out of 32) from unrelated ones. Four out of 9 young from polydactylous females were polydactylous. In the next 3 generations selected males produced increasing percentages of polydactyls from the various classes of females but even matings between perfect

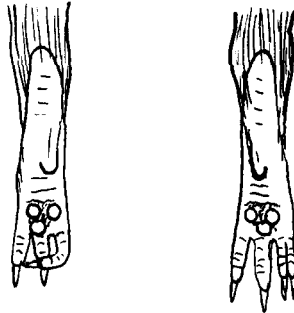


FIGURE 2.—Hind feet of an abnormal guinea pig, showing imperfect development of digits on left foot and duplication of a digit on the right foot. This type of polydactyly is to be distinguished from the atavistic type of figure 1.

polydactyls produced some normals as well as many with imperfect fourth toes. A close approach to fixation of the perfect fourth toe was reached in generation 5. Complete fixation was later attained and has continued to the present in a branch of this strain in my possession. CASTLE noted a slight excess tendency toward extra toes on the left side (630 left, 589 right).

The breeding results could be accounted for neither on the basis of a simple recessive, nor of a simple dominant. CASTLE concluded that the extra toe was probably inherited in a manner intermediate between blending and alternative inheritance and that there was some sort of latency in normal guinea pigs. In a later publication (1911) he noted that "an alternative explanation is possible that the development of the fourth toe depends upon the inheritance of several independent factors and that the more of these are present the better will the structure be developed."

STOCKARD and PAPANICOLAU (1920) concluded that the character is a Mendelian dominant. In a more recent paper (1930) however, STOCKARD reaches the conclusion that the mode of inheritance must be more complex. He has found it occurring occasionally in eight distinct stocks and considers it probable that it is latent in all stocks. The results of matings were in the main similar to those reported by CASTLE. Normal \times normal produced 11 percent (22 out of 199) with the extra toe. Conversely when both parents were four-toed, 25 percent (53 out of 214) of the progeny were normal. Contrary to CASTLE he obtained no normals among 35 young, both of whose parents had perfect fourth toes. In all cases in which both parents were of the same grade, this grade was in excess among the progeny. The proportion of four-toed young from matings in which one parent was four-toed and the other normal varied with the grade of the former and the source of the latter.

PICTET (1932) has recently reported on experiments with this character. Extra-toed animals appeared in a certain stock which had previously produced only normals, 3000 in number. The 7 pairs which produced polydactyls at all, gave a total of 9 polydactyls in 144 young. The following table gives in condensed form the results obtained in eight later generations in which both parents trace in part at least to this foundation stock.

	TOTAL	4-TOE	PERCENT 4-TOE
Normal \times Normal	196	9	4.6
Normal \times 4-toe	148	47	31.8
4-toe \times 4-toe	173	121	69.9

As in CASTLE's and STOCKARD's data, matings of polydactyls with unrelated normals gave smaller percentages of polydactyls (15 in 101 or 14.9 percent) than matings with related normals. PICTET's results resembled CASTLE's in the excess of left little toes over right ones (46 left only, 18 right only, 139 both). He could find no heredity of the asymmetry.

PICTET attempted to reach a definite factorial analysis. His reasoning may be summarized as follows: normal \times normal may give ratios approxi-

mating 15 normal to 1 polydactyl. This requires a dihybrid ratio at least. But polydactyly cannot be due merely to a combination of two recessives implied by the 15:1 ratio, since polydactyl \times polydactyl may produce normals. Thus a third gene is required. The specific hypothesis put forward is that polydactyly depends on the combination of PP with at least two positive genes from the series E, e and N, n . The six combinations $PPEENN$, $PPEENn$, $PPEEnn$, $PPEeNN$, $PPeeNN$, and $PPEeNn$ are 4-toed while the other 21 combinations are normal.

He assumes that all of the original matings of normal \times normal which gave polydactyly were of the type $PpEenn \times PpeeNn$ yielding 15 normal to 1 polydactyl as observed. Matings between polydactyls from these ($PPEeNn$) would then be expected to yield 5 normals to 11 polydactyls. The observed F_1 ratio, 9 normals to 19 polydactyls, is in agreement. The ratio of 43 normals to 102 polydactyls from all matings between polydactyls from the next 7 generations is pointed to as additional evidence, overlooking that most of the polydactyls of these generations would be homozygous in E or N or both and would give higher percentages.

In the cases of normal \times normal and normal \times polydactyl, he finds it possible to yick out formulae for the parents which will account for each type of ratio observed. It must be taken into account, however, that with 6 different genetic constitutions assigned polydactyls and 21 to normals there are 126 possible types of matings of normal with polydactyl and 210 between normals. This gives considerable room for choice of formulae to fit observed cases. Under such conditions the demonstration that a choice of formula is capable of accounting for observed ratios does not constitute proof that the formulae are correct.

The whole argument also ignores the fact already demonstrated (WRIGHT 1926) that non-genetic factors play such an important role that normals and polydactyls may be of the same genetic constitution. It may be concluded that the specific genes P, p, E, e and N, n cannot be taken seriously.

My own experiments began in a preliminary way in Dr. CASTLE's laboratory in 1914. The results showed clearly that further advance was hardly possible without the use of stocks known to be homozygous or nearly so, that is to say, of closely inbred stocks. Shortly thereafter I had an opportunity to study the records of such stocks. A considerable number of inbred lines were started at the experiment station of the U. S. Bureau of Animal Industry at Beltsville, Maryland in 1906 and maintained by brother-sister mating. Among the 23 which persisted more than a generation or two, it was found (WRIGHT 1922) that 12 had produced no polydactyls in a total of 8400 young (1906 to 1915) as shown by the records (which however merely noted the occurrence of polydactyly, not of normality during this period). Five of the strains had produced a total of 12

polydactyls in 4600 young or 0.26 percent. The remaining 6 strains had produced polydactyls in percentages ranging from 1.7 percent to 19.0 percent.

TABLE 1
Occurrence of polydactyls in 23 inbred strains.

STRAIN	4-TOED	TOTAL	PERCENT 4-TOED
1, 3, 9, 13, 15, 18, 19, 20, 21, 23, 32, 34	0	8404	0
2, 7, 14, 17, 39	12	4626	0.3
24	19	1142	1.7
36	26	1298	2.0
11	25	1151	2.2
38	59	768	7.7
35	181	1343	13.5
31	152	802	19.0

A study of the histories of these families, especially of 35 and 31, led to the conclusion that "the segregation among the family lines is so sharp that it is probable that a careful investigation of polydactyly would yield Mendelian results, though much nongenetic variation must be present."

Since 1916 the number of digits on the hind foot and the grade of extra toe if present was made a matter of routine recording for every animal born in the colony of the Bureau of Animal Industry. The positive notation of normality doubtless increased the reliability. A large branch of family 35, descended from a single mating in the 12th generation of brother-sister mating, was made the object of intensive study with regard to non-genetic factors. Important effects of age of parents (presumably of dam) and of season of birth were demonstrated and it was shown that these constituted only a part of the total non-genetic variability (WRIGHT 1926). In the same paper the results of crosses between certain of the strains (2, 13, 32, 35) and Professor CASTLE's strain of perfect polydactyls [*D*] were reported briefly as indicating "segregation of two or three major genetic factors, the results being clearly different in crosses with different 3-toed stocks."

The present paper will present more fully than before and on the basis of additional data, the analysis of the variability within the inbred strain No. 35. It is hoped to present in detail in later papers the results of the crosses between inbred strains referred to above, the results of linkage tests and the results in a strain (I) in which pollex and hallux appear as well as little toe.

DIFFERENTIATION OF SUBSTRAINS OF FAMILY 35

The branching lines of descent from the foundation mating of family 35 are shown in figure 3 and table 1. Four branches (B, C, E and G) started

in the second generation. Three of these produced low percentages of 4-toed young, while one (C, D) produced none at all among 335 young in a history extending through the 12th generation. One of the other branches of the 2nd generation (E) split off a branch (F) in the third generation which never produced polydactyls. These early differences may easily have been the result of segregation of factors heterozygous in the original pair.

Of greater interest are the records of the later branches, all descended from a single mating in the 12th generation of brother-sister mating. Under such mating, some combination of genes should have become nearly fixed

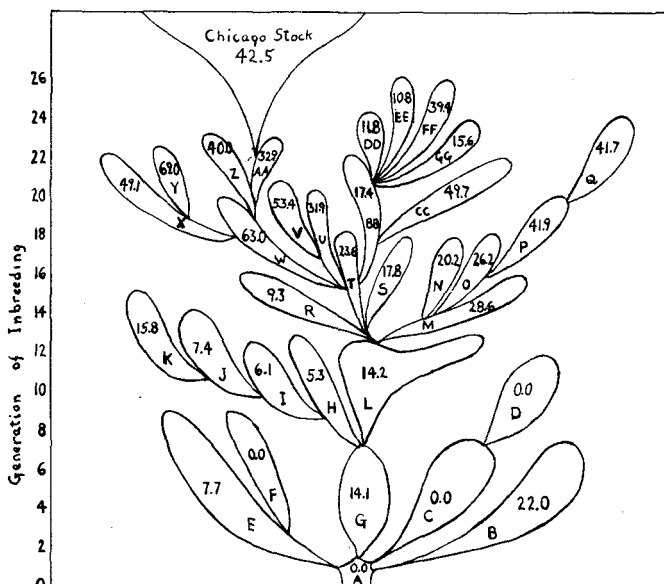


FIGURE 3.—Branches of family 35 showing percentages of polydactyly.

by the 12th generation, assuming of course the absence of new mutations or selection for heterozygosis. The theoretical rate of decrease of heterozygosis is 19.1 percent per generation, under which, 92 percent of the genes, not similarly homozygous in the foundation pair of guinea pigs, should have become homozygous. The percentage of unfixed genes should be approximately halved with each additional 3 generations of brother-sister ancestry.

All of the 21 substrains, descended from the single mating in the 12th generation, indeed nearly all of the matings, have produced polydactyls. The mere occurrence of both 3-toed and 4-toed young would indicate continued heterozygosis on such an interpretation as that of PICTET. It does not necessarily do so, however, if non-genetic factors play a role. The difference in percentage among the strains (9 percent to 69 percent) are, however, indicative of real genetic differentiation.

TABLE 2
Percentage of polydactyls in different branches of family 35.

BRANCHES			MATINGS	YOUNG	
SYMBOL	FIRST MATING	PARENT BRANCH	NUMBER	NUMBER	PERCENT 4-T
A	0-1	—	2	18	0.0
B	2-3	A	13	109	22.0
C	2-2	A	19	152	0.0
D	8-6	C	17	183	0.0
E	2-4	A	13	117	7.7
F	3-12	E	7	88	0.0
G	2-1	A	11	99	14.1
H	8-1	G	12	152	5.3
I	9-1	H	8	98	6.1
J	10-2	I	18	215	7.4
K	11-13	J	9	76	15.8
L	8-2	G	14	148	14.2
M	13-3	L	5	49	28.6
N	14-3	M	6	104	20.2
O	14-7	M	9	122	26.2
P	16-3	O	10	124	41.9
Q	20-14	P	7	60	41.7
R	13-7	L	5	43	9.3
S	14-12	R	14	157	17.8
T	14-5	R	10	123	23.6
U	16-4	T	6	69	31.9
V	17-7	U	5	58	53.4
W	16-7	T	5	54	63.0
X	18-2	W	5	53	49.1
Y	19-5	X	11	84	69.0
Z	19-3	W	9	100	40.0
AA	20-17	Z	10	79	32.9
BB	16-9	T	10	121	17.4
CC	18-7	BB	16	167	49.7
DD	21-3	BB	15	136	11.8
EE	21-4	BB	12	130	10.8
FF	21-8	BB	8	66	39.4
GG	21-18	BB	9	77	15.6

Comparison of the observed frequencies of 3-toed and 4-toed young in the 21 substrains with those calculated from the percentages in the grand total (31.07 percent 4-toed) yields a X^2 of 247. The probability that such a value of X^2 could arise by random sampling (20 degrees of freedom) is indefinitely small. There may, however, be some other factor than heredity tending to cause correlated occurrence within a substrain. It is shown later that there is an important correlation between litter-mates which is not

genetic in origin. But even if litter-mates were invariably identical with respect to number of digits, so that number of litters instead of number of individuals should be used in calculating X^2 , the value of the latter would still be as great as 101 (247 divided by 2.44 the mean size of litter) and this still has no appreciable chance of arising by random sampling. There seems to be no other factor which brings about correlated occurrence to an important extent and as X^2 could be reduced to 50 and have a chance of only about .0002 of origin by random sampling, there can be no doubt of the reality of the genetic differentiation among the substrains.

On comparing each substrain with that from which it was derived, it will be seen that there has been some tendency toward persistence. The correlation between successive substrains is +.47. There are at least half a dozen cases, however, of changes of percentages which appear important. Their interpretation as due to segregation is rather unlikely, in view of the many generations of brother-sister mating back of some of the most striking ones. The most plausible explanation seems to be the occasional occurrence of minor mutations followed by segregation.

DEGREE OF DETERMINATION BY HEREDITY

It is of interest to determine the portion of the total variability due to substrain differentiation. But at once we encounter the difficulty that the character does not occur in grades from which the variance can be calculated directly. The most significant classification is merely the dichotomy 3-toed or 4-toed.

It is already clear that this dichotomy cannot correspond to alternative phases of a single factor. It is the result of a physiological threshold in a character affected by many factors. It is therefore reasonable to assume that there is a scale of factor combinations to which each factor makes a fairly constant contribution and that variabilities may be compared on such a scale. As the unit of measurement it is convenient to take the standard deviation within a substrain ($\sigma_s = 1$). The deviation of the mean (m) of each substrain from the threshold for polydactyly can be found from a table of probability integrals on the assumption that the distribution within each group is normal on such a scale as described. Thus for the deviation of the mean of the group, $m = \text{prf}^{-1}(q - \frac{1}{2})$ where q is the proportion above the threshold and prf^{-1} is the inverse probability integral,

where $\text{prf } x = \int_0^x \frac{e^{-z^2/2} dz}{\sqrt{2\pi}}$. The variance of such means can now be found

by the usual formula

$$\sigma_m^2 = \frac{\Sigma(m - \bar{m})^2 f}{n} = \frac{\Sigma m^2 f}{n} - \bar{m}^2$$

where \bar{m} is the weighted average of the means, $\left(\bar{m} = \frac{\sum mf}{n}\right)$. If the set of substrains are thought of as a random sample from a large number, correction should be made for the uncertainty of the grand average (\bar{m}) by multiplying by $\frac{h}{h-1}$ where h is the number of substrains (assumed to be of approximately the same size). If, on the other hand, the groups be thought of, not as a sample, but as including *all* subdivisions of the population, no such correction should be made.

In the present case the question at issue is the proportion of the variance of the actual population due to the differentiation of its substrains, rather than proportion in a hypothetical population made up of an indefinite number of such substrains. This correction is accordingly not made in the final estimate. It may be noted that it is even less justified in cases analyzed later, such as subdivision by age of mother and month of birth.

Another correction is for the variance of means due merely to accidents of sampling within the substrains. The formula for the variance of means of groups consisting of f individuals is $\frac{1}{f} \left(= \frac{\sigma_s^2}{f} \right)$. The value $\frac{h}{n}$ may be taken, if the groups are approximately the same size. In the present case this correction is not very important $\left(\frac{h}{n} = .0106 \right)$.

The variance of the total population is the sum of the variances within groups and of group means:

$$\sigma_T^2 = \sigma_s^2 + \sigma_m^2 = 1 + \sigma_m^2.$$

The proportion of the total variance due to substrain differentiation is thus $\frac{\sigma_m^2}{1 + \sigma_m^2}$. This does not differ appreciably, in most cases, from PEAR-

SON's formula for squared biserial eta, but does not involve the assumption that the total distribution is normal.

For the 21 substrains of table 3 the above formula gives 19.2 percent if no corrections are made, 18.5 percent if correction is made for average size of subgroup and 19.3 percent if both corrections are made. The second of these may be taken as the best estimate of the proportion of the variance of the actual population due to differentiation of its substrains.

This differentiation of substrains with respect to polydactyly contrasts with the relative uniformity of the same substrains in most other respects

(WRIGHT and EATON 1929). The family is characterized for example by a tricolor pattern. The percentage of white varies in individuals from 5 percent to 100 percent but the substrains derived from the same mating in the 12th generation vary only from 60 to 73 percent and wholly at random. The parent offspring correlation of only $+ .024 \pm .013$ with respect to amount of white confirms the view that in this respect no appreciable portion of the variability since the 12th generation is genetic.

THE CHICAGO STOCK

The data above refer to the history of family 35 from a mating in the 12th generation from 1916 to 1924 inclusive. A small stock has been maintained in Chicago from 1926 to the present. It has not been practicable to continue the system of mating brother with sister but the entire stock traces to a single Beltsville mating in the 22nd generation of inbreeding. This Chicago branch has produced 152 polydactyls among 358 young through June 1933, an average of 42.5 percent. This does not differ very much from the record of 32.9 percent produced by the Beltsville sub-strain (AA) from which it came and still less from the next most closely allied Beltsville substrain (Z with a record of 40.0 percent).

It may be noted that the Beltsville records were made wholly as a matter of routine, no greater attention being paid to strain 35 than to any other. No statistical analysis was started until after the records through 1924 were complete. The Chicago data on the other hand were recorded with especial care after such an analysis had been made on the Beltsville data.

CORRELATION BETWEEN PARENT AND OFFSPRING

In the Beltsville stock (table 3, next to last column) 3-toe \times 3-toe produced 26.6 percent polydactyls, while 4-toe \times 4-toe produced 41.6 percent. This does not indicate much genetic difference between normals and polydactyls within the stock, perhaps no more than expected on the basis of the substrain differences demonstrated above.

To test this matter, the results of matings should be compared *within* substrains or within groups of similar substrains. The data are divided into four such groups (I with 9–16 percent, II with 17–29 percent, III with 32–42 percent and IV with 49–69 percent polydactyls respectively) in table 3. The last column gives the average percentage from each type of mating on weighting the percentage within each group from that type of mating by the total number of young from that group.

It appears that *within* groups, 4-toe \times 4-toe produced on the average even fewer polydactyls than did 3-toe \times 3-toe (27.2 percent compared with 30.6 percent) but the difference has no significance. The result from

TABLE 3
Results of different types of matings within 4 subdivisions of family 35. Number of matings (Mat.), number of young (Y) and percentage of polydactyls (percent 4-T) are recorded.

DAM	SIRE	GROUP I (9 PERCENT-16 PERCENT) MAT. Y. PERCENT 4-T			GROUP II (17 PERCENT-29 PERCENT) MAT. Y. PERCENT 4-T			GROUP III (32 PERCENT-42 PERCENT) MAT. Y. PERCENT 4-T			GROUP IV (49 PERCENT-69 PERCENT) MAT. Y. PERCENT 4-T			TOTAL			
		MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	MAT.	Y.	PERCENT 4-T	
3-toe	3-toe	31	269	14.9	35	414	20.3	23	204	36.3	12	132	55.3	101	1019	26.6	30.6
3-toe	4-toe	4	31	9.7	8	74	33.8	6	49	49.0	5	31	71.0	23	185	40.0	40.8
4-toe	3-toe	6	79	3.8	8	114	20.2	3	35	40.0	2	42	47.6	19	270	22.2	27.8
4-toe	4-toe	2	7	0.0	5	74	17.6	17	210	37.6	23	211	55.5	47	502	41.6	27.2
Total		43	386	11.9	56	676	21.5	49	498	38.4	42	416	55.8	190	1976	31.1	

3-toe×4-toe diverges considerably from the others but depends on too few matings to be given much importance. It is clear that the factors responsible for differences in the number of toes among animals of the same substrain must be practically wholly non-genetic.

Returning to the total population, it is of interest to find the correlation between parent and offspring. Tetrachoric coefficients were obtained by PEARSON'S formula from the totals of table 3, based on 1976 offspring but only 190 matings. (Most of the tetrachoric correlations given in this paper were calculated from very convenient graphs prepared by my colleagues Prof. L. L. THURSTONE and LEONE CHESTRE in mimeographed form.) The assumption is again made that there is a graded scale of factor combinations back of the alternative categories, normal and polydactyl. Similar correlations were obtained from each of the four subdivisions of the data and averaged.

CORRELATION	TOTAL POPULATION	AVERAGE OF 4 SUBDIVISIONS
Offspring with dam	+ .106	— .102
Offspring with sire	+ .261	+ .086
Average	+ .183	— .008

It is again brought out that there is no genetic variability *within* the substrains. Even in the total population environmental differences are clearly much more important than genetic ones.

With homozygosis within strains, a correlation of +.183 between parent and offspring indicates that 18.3 percent of the variance of the total stock (on the scale of factor combinations) is due to hereditary differences and 81.7 percent to environmental ones. This agrees well with the estimate of 18.5 percent of the variance as due to substrain differentiation previously reached.

The results in the Chicago stock, not included in the above, are given in table 4 below.

TABLE 4
Results from different types of matings in Chicago stock.

DAM	SIRE	MATINGS	YOUNG	PERCENT 4-TOE
3-toe	3-toe	24	140	41.4
3-toe	4-toe	13	71	29.6
4-toe	3-toe	11	70	41.4
4-toe	4-toe	16	77	57.1
		64	358	42.5

There is a slight indication of hereditary difference between 3-toed and 4-toed animals but the number of matings is too small to attribute much significance to it. It is interesting that the tetrachoric correlation between dam and offspring (+.19) is here greater than that between sire and offspring (+.04) which is the opposite of the situation in the Beltsville stock. The average parent-offspring correlation (+.11) is not certainly significant even on the basis of a standard error calculated from number of offspring. Taken at face value, however, it would indicate about the same proportion of the variance to be genetically determined as in the Beltsville data since under *random* mating a correlation of +.11 between parent and offspring implies 22 percent determination by heredity. We shall return to this question later.

CORRELATION BETWEEN LITTER-MATES

Most tangible environmental factors should act alike on litter-mates. If such factors are important there should be a tendency for litters to consist wholly of 3-toed or wholly of 4-toed young. Table 5 shows an analysis

TABLE 5

The distribution of polydactyls in litters of 2 or more in the Beltsville and Chicago stocks. The theoretical values (Calc.) are derived from binomial expansions based on the percentage of polydactyls in each group.

SIZE OF LITTER		NUMBER OF 4-TOE IN LITTER						NUMBER OF LITTERS	NUMBER OF YOUNG	PERCENT OF 4-T
		0	1	2	3	4	5			
Beltsville 2	Obs.	146	76	64	—	—	—	286	572	35.7
	Calc.	118.4	131.2	36.4	—	—	—			
	Diff.	+27.6	-55.2	+27.6	—	—	—			
Beltsville 3	Obs.	119	63	34	46	—	—	262	786	34.2
	Calc.	74.6	116.4	60.5	10.5	—	—			
	Diff.	+44.4	-53.4	-26.5	+35.5	—	—			
Beltsville 4	Obs.	44	23	11	10	5		93	372	25.5
	Calc.	28.6	39.2	20.2	4.6	0.4				
	Diff.	+15.4	-16.2	-9.2	+5.4	+4.6				
Beltsville 5	Obs.	9	6	0	1	0		16	80	11.3
Beltsville 6	Obs.	1	0	0	1	0		2	12	25.0
Chicago 2	Obs.	26	14	9				49	98	32.7
	Calc.	22.2	21.6	5.1						
	Diff.	+3.8	-7.6	+3.9						
Chicago 3	Obs.	16	11	12	13			52	156	47.1
	Calc.	7.6	20.4	18.4	5.6					
	Diff.	+8.4	-9.4	-6.4	+7.4					
Chicago 4	Obs.	3	4	4	3	1		15	60	35.4
Chicago 5	Obs.	0	1	0	0	1	1	3	15	66.7

of litters of 2, 3 and 4 in the Beltsville stock from this standpoint. The litter sizes are those after deducting unclassified young (feet eaten when found). The observed frequencies (Obs.) of litters containing no polydactyls, one, two, et cetera are compared with frequencies (Calc.) expected on the assumption of random occurrence in litters. Litters of 2 contained 35.7 percent polydactyls. If there were no tendency to concurrence in litters, the chance of occurrence of 0, 1 and 2 polydactyls (D) respectively should be according to the terms of the expression $(.643 N + .357 D)^2$. Similarly in litters of 3, there were 34.2 percent polydactyls and the frequency of different sorts of litter should be in accordance with the terms of $(.658 N + .342 D)^3$. In litters of 4 there were 25.5 percent polydactyls and we expand $(.745 N + .255 D)^4$ to find the expected proportions.

It will be seen that there was a marked excess of litters wholly normal or wholly polydactyl with corresponding defect of mixed litters (except in the case of 3 polydactyls in litters of 4).

A similar analysis is presented of the less numerous Chicago data for litters of 2 and 3. Here again there is an excessive number of litters which consist wholly of one or the other sort of young and a deficiency of mixed litters. The χ^2 method can be used to test the significance of these differences. Two degrees of freedom are lost in each case through acceptance of the total numbers and the total percentages of polydactyls in each size of litter. Because of small numbers, litters with 3 and with 4 polydactyls are combined in the case of litters of 4 in the Beltsville data.

	LITTER SIZE	χ^2	n	PROB.
Beltsville	2	50.6	1	.000,000
	3	182.5	2	.000,000
	4	39.2	2	.000,000
Chicago	2	6.3	1	.012
	3	25.6	2	.000,000

There is obviously no doubt of the reality of the tendency to concurrence of polydactyly in litter-mates. This contrasts with the low fraternal correlation with respect to white spotting in the same material and the absence of any tendency toward concurrence of otocephaly in another in-bred family.

It is interesting to throw this result into the form of the correlation between litter-mates. This can be done by making up 2×2 tables from the data and calculating the tetrachoric coefficients of correlation. Standard errors have been calculated by PEARSON'S formula quoted by KELLEY (1923, p. 258) multiplied by a term to allow for the repetition of indi-

viduals in litters larger than 2, namely, $\sqrt{\frac{1+(L-1)r}{1+r}}$ where L is size of litter.

BELTSVILLE DATA			CHICAGO DATA		
SIZE OF LITTER	r_{00}	σ_r	SIZE OF LITTER	r_{00}	σ_r
2	.62	$\pm .07$	2	.53	$\pm .19$
3	.67	$\pm .05$	3	.60	$\pm .13$
4	.53	$\pm .11$	4,5	.38	$\pm .21$
5,6	.25	$\pm .28$			
Average	.623	$\pm .040$	Average	.54	$\pm .10$

The correlations are averaged by the formula

$$\frac{\Sigma(r/\sigma_r^2)}{\Sigma(1/\sigma_r^2)} \pm \sqrt{\frac{1}{\Sigma(1/\sigma_r^2)}}$$

Thus 62 percent of the variability in the Beltsville data and 54 percent in the Chicago data is determined by factors common to litter-mates. The difference is not significant but as far as it goes it indicates less genetic variability in the Chicago data. In the Beltsville data, all of the substrain differentiation amounting to 18 percent of the total variance is of course included in that common to litter-mates. The variance common to litter-

mates would be 54 percent $\left(= \frac{62-18}{100-18} \right)$ within a homozygous group or exactly that found in the Chicago data.

In the Chicago data comparisons have also been made between animals from different litters from the same mating. The results for successive litters and for litters two or more apart are shown below.

TABLE 6

Correlations between individuals of different litters from the same mating. Chicago stock.

		Following		
earlier		3-T	4-T	Total
	3-T	200	78	278
	4-T	85	66	151
	Total	285	144	429

$r = +.25$

TABLE 7

Correlations between individuals of different litters from the same mating. Chicago stock.

		2 or more later		
earlier		3-T	4-T	Total
	3-T	147	44	191
	4-T	124	81	205
	Total	271	125	396

$r = +.29$

The standard errors on the assumption of no correlation are .080 and .082 respectively. There is thus no doubt of the reality of these correlations. The average value .27 is just half that between litter-mates in the same material, indicating that factors which act alike on litter-mates but not on sibs from different litters, and factors which act alike on all offspring from the same mating contribute equally to variance in this stock in which there is little or no genetic variability. The parent-offspring correlation of .11 would indeed indicate a genetic fraternal correlation of .11 contributing to the .27 found above, but as will be brought out more fully later this figure is certainly too high. As to the nature of this group of factors, effects of the condition of the mother over long periods seem most probable.

VARIABILITY OF DIGITS OF ONE FOOT

All authors who have studied polydactyly of guinea pigs, have noted its frequent asymmetry. This in itself indicates an important element of variability which is not hereditary (WRIGHT 1916). The tables below show the relations between the left and right feet of the same animals in the Beltsville and Chicago records; applying the grades "poor" and "good" to single toes.

TABLE 8

Correlation between right and left feet of individuals.

		Right			
		3-T	POOR 4-T	GOOD 4-T	TOTAL
Left	3-T	1362	96	33	1491
	Poor 4-T	149	101	34	284
	Good 4-T	50	55	96	201
Total		1561	252	163	1976

TABLE 9

		Right			
		3-T	POOR 4-T	GOOD 4-T	TOTAL
Left	3-T	206	26	3	235
	Poor 4-T	34	23	13	70
	Good 4-T	13	16	22	51
Total		253	65	38	356

Beltsville
r = +.78

Chicago
r = +.73

On reducing these to 2x2 tables by grouping "poor" and "good" together, the tetrachoric correlations come out $+ .779 \pm .015$ in the Beltsville data and $+ .73 \pm .05$ in the Chicago data. The correlation within a homozygous substrain of the Beltsville stock should be about

$$.73 \left(= \frac{.78 - .18}{1 - .18} \right),$$

exactly that found in the Chicago data. This is another

indication that genetic variability was practically lacking in the latter.

It is interesting to compare this correlation with that between *single* hind feet of litter-mates. The data below do this for 322 litter-mates of the Chicago data.

TABLE 10

TABLE 11

TABLE 12

The correlations between single feet of litter-mates of Chicago data.

Left				Right				Right						
	3-T	4-T	TOTAL		3-T	4-T	TOTAL		3-T	4-T	TOTAL			
Left	3-T	159	45	204	Right	3-T	337	72	409	Left	3-T	179	41	220
	4-T	46	72	118		4-T	104	131	235		4-T	42	60	102
	Total	205	117	322		Total	441	203	644		Total	221	101	322
$r = +.58$				$r = +.59$				$r = +.61$						

There is obviously no special tendency toward resemblance of feet of the same side. The average, $+ .59$, is surprisingly close to the correlation between the two feet of the same animal ($+ .73$). Of the factors determining the condition of one foot, 27 percent act on that foot alone, 14 percent act on both feet of the individual but not on litter-mates, while 59 percent act alike on litter-mates.

Correlations of $+ .59$ between single feet of litter-mates and of $+ .73$ between feet of the same individual imply a correlation of $+ .68$ ($= \frac{4 \times .59}{2 \times 1.73}$) between the total factor complexes of litter-mates considered as the sum of those for the separate feet. The observed value $+ .54$ is smaller probably because of lack of linearity in the relations of the factors for single toes and the scale for individuals based on the presence of at least one extra toe.

One point that comes out of both the Beltsville and Chicago data is the slightly greater frequency of little toes on the left side. Combining there were 26.0 percent on the left side and 22.2 percent on the right side. The difference is about 3 times its standard error. Both CASTLE and PICTET found an excess number of left digits.

SEX

There is no relation to sex. In the Beltsville data the percentage of polydactyls was 31.1 percent in the males and exactly the same to the first decimal place in females. In the Chicago data, there were 41.1 percent among the males and 42.4 percent among the females. This lack of difference contrasts with the important sex differences among otocephalic monsters and in amount of white spotting.

AGE OF DAM

With a large proportion of the variability common to litter-mates and only about half of this common to non-litter-mates from the same mating, it should be possible to find some indication from the records of the nature of the more important factors.

The most important relation to be found was one which was quite unexpected, namely with age of mother. Table 13 gives the results by months. The Beltsville inbred stock (1976 animals) is here combined with data from some selection experiments at Beltsville in which animals from family 35 were mated *inter se* without regard to relationship (246 animals) and with the Chicago data (358 animals). Figure 4 shows the percentage of polydactyls by month to 14 months and by such groupings of months thereafter as to keep the number in each group above 100. There is a rapid drop from the 83 percent and 60 percent produced by 3 and 4 month old females respectively to the 17 percent produced on the average by all females over 15 months of age. There is no question of the statistical significance by any method of calculation.

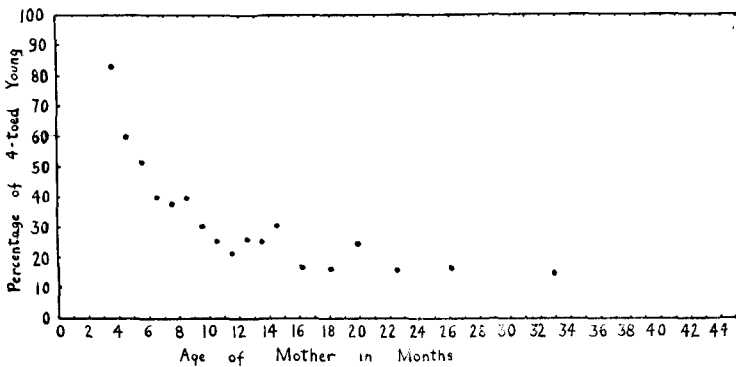


FIGURE 4.—Percentage of 4-toed young according to age of mother. All points except first (3 months) based on more than 100 young.

Furthermore, among the young which are 4-toed at all, the percentage of good polydactyls decreases rapidly with increasing age of the mother.

It is desirable to find whether these age effects are found in subdivisions of the family which differ markedly in total percentage of polydactyly. Tables 14–20 show the distributions in the same four divisions of the Beltsville inbred stock used previously, in the crossbred Beltsville stock, and in the Chicago stock as well as in the grand total, each by four age groups. In every case there is a significant downward trend of polydactyly in relation to age of mother. The squared correlation η^2 either for the Beltsville inbreds or the total is about .13, indicating 13 percent determination of the total variance by age of dam.

The effect has been presented as of age of mother rather than of father, but the Beltsville data give little basis for a distinction. Almost all of the brother-sister matings were between litter-mates and most of those in the miscellaneous group were between animals of nearly the same age. The exceptional cases however pointed to age of dam.

TABLE 13
Occurrence of polydactyly in relation to age of mother. Age 3 means from 3 months to 4 months, etc.

	AGE OF DAM IN MONTHS																
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
3-toe	2	107	82	99	108	108	98	111	108	99	77	105	48	77	75		
Poor 4-toe	3	123	71	56	55	65	41	30	21	35	27	42	6	19	8		
Good 4-toe	7	39	16	10	11	7	2	8	8	1	—	6	—	—	—		
Total	12	269	169	165	174	180	141	149	137	135	104	153	54	96	83		
Percent 4-Toe	83.3	60.2	51.5	40.0	37.9	40.0	30.5	25.5	21.2	26.7	26.0	31.4	11.1	19.8	9.6		

	AGE OF DAM IN MONTHS													
	18	19	20	21	22	23	24	25	26	27	28	29	30	31
3-toe	74	49	29	44	23	37	35	23	19	17	14	16	18	4
Poor 4-toe	17	9	15	9	5	3	7	6	3	5	1	3	4	2
Good 4-toe	4	2	—	1	—	2	—	—	—	—	—	—	—	1
Total	95	60	44	54	28	42	42	29	22	22	15	19	22	7
Percent 4-Toe	22.1	18.3	34.1	18.5	17.9	11.9	16.7	20.7	13.6	22.7	6.7	15.8	18.2	42.9

	AGE OF DAM IN MONTHS													TOTAL
	32	33	34	35	36	37	38	39	40	45				
3-toe	17	3	7	5	11	4	1	2	1	1	—	—	1758	
Poor 4-toe	3	—	—	1	1	—	—	1	—	—	—	—	697	
Good 4-toe	—	—	—	—	—	—	—	—	—	—	—	—	125	
Total	20	3	7	6	12	4	1	3	1	1	—	—	2,580	

TABLES 14-20

Correlation of polydactyly with age of mother.

TABLE 14
Group I. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	53	75	104	108	340
Poor 4-toe	20	6	10	6	42
Good 4-toe	2	0	1	1	4
Total	75	81	115	115	386
Percent					
4-toe	29.3	7.4	9.6	6.1	11.9

$\eta^2 = .11, \eta = .32$

TABLE 15
Group II. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	68	89	157	217	531
Poor 4-toe	28	33	40	30	131
Good 4-toe	8	2	4	0	14
Total	104	124	201	247	676
Percent					
4-toe	34.6	28.2	21.9	12.1	21.4

$\eta^2 = .07, \eta = .27$

TABLE 16
Group III. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	29	41	106	131	307
Poor 4-toe	48	43	38	31	160
Good 4-toe	14	6	5	6	31
Total	91	90	149	168	498
Percent					
4-toe	68.4	54.4	28.9	22.0	38.4

$\eta^2 = .18, \eta = .43$

TABLE 17
Group IV. Beltsville.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	15	29	73	67	184
Poor 4-toe	47	51	59	28	185
Good 4-toe	17	15	14	1	47
Total	79	95	146	96	416
Percent					
4-toe	81.0	69.5	50.0	30.2	55.8

$\eta^2 = .20, \eta = .45$

TABLE 18
Beltsville crossbred.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	18	41	84	47	190
Poor 4-toe	21	12	12	4	49
Good 4-toe	3	3	1	0	7
Total	42	56	97	51	246
Percent					
4-toe	57.1	26.8	13.4	7.8	22.7

$\eta^2 = .21, \eta = .45$

TABLE 19
Chicago.

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	8	40	74	84	206
Poor 4-toe	33	31	36	30	130
Good 4-toe	18	2	1	1	22
Total	59	73	111	115	358
Percent					
4-toe	86.4	45.2	33.3	27.0	42.5

$\eta^2 = .25, \eta = .50$

TABLE 20
Grand Total

	AGE OF DAM (MONTHS)				TOTAL
	3-6	6-9	9-15	15-	
3-toe	191	315	598	654	1758
Poor 4-toe	197	176	195	129	697
Good 4-toe	62	28	26	9	125
Total	450	519	819	792	2580
Percent 4-toe	57.6	39.3	27.0	17.4	31.9
Percent good of 4-toe	23.9	13.7	11.8	6.5	15.2
Percent good of total	13.8	5.4	3.2	1.1	4.8

$$\eta^2 = .134, \eta = .366$$

In the Chicago experiments an especial effort was made to mate young females with old males and *vice versa*. Table 21 shows the results classified in 4 age groups for each parent. A relation to age of mother comes out as definitely as in the Beltsville data ($\eta = .50$). The slight downward

TABLE 21
Occurrence of 3-toed and 4-toed young in relation to ages of sire and of dam. Chicago data.

AGE OF SIRE	AGE OF DAM								TOTAL		
	3-6		6-9		9-15		15-37		3-T	4-T	PERCENT 4-T
	3-T	4-T	3-T	4-T	3-T	4-T	3-T	4-T			
3-6	0	10	2	2	6	2	8	4	16	18	52.9
6-9	2	4	4	12	7	6	18	7	31	29	48.3
9-15	4	18	20	8	30	9	20	9	74	44	37.3
15-37	2	19	14	11	31	20	38	11	85	61	41.8
Total	8	51	40	33	74	37	84	31	206	152	42.5
Percent 4-toe	86.4		45.2		33.3		27.0		42.5		

trend in relation to age of sire in part reflects a slight correlation with age of dam present even in this data. It is not statistically significant. It is probable the entire effect is one of age of dam.

Table 22 shows the percentage of 4-toed young in each of the 4 subdivisions of the Beltsville data in relation to the age of the grandparents at birth of the parents (or dam in the few cases in which the parents were not litter-mates). There appears to be a slight downward trend of the percentage in the totals (next to last column) but this practically disappears (last column) on correcting for the irregularities in distribution among the 4 groups by weighting the percentages by the totals for each group. Correction for age of dam was tried but made no appreciable change in the re-

TABLE 22
Occurrence of polydactyly in relation to age of dam's parents. Beltsville.

AGE OF GRANDAM	SUBSTRAIN (BELTSVILLE)								TOTAL		
	I		II		III		IV		NUM- BER	PERCENT 4-T	WEIGHTED PERCENT 4-T
	NUMBER 4-T	PERCENT 4-T	NUMBER 4-T	PERCENT 4-T	NUMBER 4-T	PERCENT 4-T	NUMBER 4-T	PERCENT 4-T			
3-5	94	7.4	174	25.3	128	43.0	129	58.1	525	34.5	33.2
6-8	91	13.2	106	14.2	88	38.6	114	49.1	399	29.3	27.5
9-14	69	14.5	211	22.7	167	38.3	122	59.0	569	34.1	32.7
15-45	132	12.9	185	20.5	115	33.0	51	56.9	483	25.3	29.8
Total	386	11.9	676	21.4	498	38.4	416	55.8	1976	31.1	31.1

sult. There is thus no reliable indication of any persistence of the effect beyond the immediate progeny of immature females. A direct physiological effect of age of mother on the egg or embryo is indicated.

TABLE 23
Polydactyly (D) in relation to age of dam (A) and birth rank (B) in Beltsville data.

BIRTH RANK	AGE OF DAM												TOTAL		
	3, 4		5		6-9		9-15		15-21		21-45		NUM- BER	PER- CENT 4-T	
	NUM- BER	PER- CENT 4-T	NUM- BER	PER- CENT 4-T	NUM- BER	PER- CENT 4-T	NUM- BER	PER- CENT 4-T	NUM- BER	PER- CENT 4-T	NUM- BER	PER- CENT 4-T			
1	212	57.5	137	45.3	72	51.4	—	—	—	—	—	—	—	421	52.5
2	—	—	—	—	317	37.5	72	25.0	—	—	—	—	—	389	35.2
3	—	—	—	—	1	0.0	304	30.3	20	5.0	—	—	—	325	28.6
4, 5	—	—	—	—	—	—	235	26.0	226	19.9	18	0.0	—	479	22.1
6-12	—	—	—	—	—	—	—	—	84	17.9	278	15.1	—	362	15.7
Total	212	57.5	137	45.3	390	40.0	611	28.0	330	18.5	296	14.2	—	1976	31.1

There is a possibility that the effect may be one of birth rank rather than age of dam, since these were closely correlated. Table 23 shows the relations in the Beltsville stock. There is a somewhat closer relation of polydactyly to age of dam ($\eta_{DA} = -.360 \pm .027$) than to parity ($\eta_{DB} = -.343 \pm .027$) with a correlation of $+.922 \pm .034$ between age and parity. The correlation of polydactyly with parity is reduced to the insignificant figure $-.030 \pm .030$ by holding age of dam constant while the correlation with age remains significant ($-.121 \pm .030$) (correcting an error in a previously published figure, WRIGHT 1926). It is concluded that parity has no effect on occurrence of polydactyly by itself.

TABLE 24
Occurrence of 3-toed and 4-toed young in relation to age and weight of dam.

WEIGHT OF DAM (GRAMS)	AGE OF DAM (CHICAGO)								TOTAL		
	3-6		6-9		9-15		15-37		3-T	4-T	PERCENT 4-T
	3-T	4-T	3-T	4-T	3-T	4-T	3-T	4-T			
400	0	2							0	2	61.5
450	1	2	1	2	3	1			5	5	
500	3	15	3	0	2	0	2	2	10	17	47.5
550	2	12	12	6	5	1	2	0	21	19	
600	1	18	9	5	12	7	9	2	31	32	50.8
650			8	6	20	8	12	3	40	17	29.8
700			2	9	29	11	26	9	57	29	33.7
750			2	2	3	4	15	4	20	10	37.8
800					0	5	3	1	3	6	
850							5	1	5	1	
Total	7	49	37	30	74	37	74	22	192	138	41.8
Percent 4-toe	87.5		44.9		33.3		22.9				

It is possible that the weight of the dam is more important than her age. The weights at the time of birth of the young were taken in the Chicago data. The relation to polydactyly and to age of dam are shown in table 24. It is evident from the marginal totals that the occurrence of polydactyly is much less closely correlated (negatively) with weight ($r = -.23 \pm .07$) than with age ($r = -.53 \pm .05$). The correlation between weight and age of dam is $+.58$ and the partial correlation of polydactyly with weight, for constant age comes out with reversed sign $+.11 \pm .07$. On the whole it is the heavier (and hence more rapidly growing) females of a given age class, rather than the lighter ones which produce the highest percentage of polydactyls although the relation is of doubtful significance.

TABLE 25

The averages of various characters at different ages of the dam. The standard deviation of size of litter was 1.01, for birth weight 16.5 reduced to 12.8 for constant size of litters (correlation $-.63$), for weaning weight 45.5 reduced to 38.5 for constant size of litter (correlation $-.53$), and for percentage of white 18.9 percent.

AGE OF DAM	NUMBER	PERCENT 4-T	LITTER SIZE	PERCENT ♂	PERCENT BORN DEAD	PERCENT DIED OF LIVE BORN	BIRTH WEIGHT	33-DAY WEIGHT	PERCENT WHITE MALES	PERCENT WHITE FEMALES
3-6	349	52.7	2.45	53.3	27.8	22.5	79.9	221.6	56.3	60.5
6-9	390	40.0	2.37	50.8	20.2	21.2	79.8	225.0	59.5	67.6
9-12	319	29.2	2.63	48.8	18.6	20.5	80.9	220.9	60.6	66.5
12-15	292	26.7	2.41	54.7	19.4	17.8	83.0	228.3	61.3	70.6
15-21	330	18.5	2.52	54.0	21.3	23.3	80.2	224.7	63.2	69.6
21-45	296	14.2	2.30	49.2	24.1	19.4	83.5	241.1	66.9	73.3
Total	1976	31.1	2.44	51.8	22.0	20.9	81.0	226.5	61.2	67.8

It seemed likely that a study of other effects of the age of the mothers might throw light on the unexpected relation to percentage of polydactyly. Table 25 presents a summary of the principal results in 6 age classes chosen to include approximately equal numbers. Except for an equally unexpected effect on the percentage of white in the young, there is remarkably little indication of age effects. Size of litter is at its highest in females which are approaching or have just reached maturity and lowest in the oldest group. There is a decreasing percentage of stillborn young with increasing maturity but a rise in the oldest group makes this difficult to relate to polydactyly. Mortality between birth and weaning shows nothing significant. The birth weight of young born alive and weaning weight (both corrected for litter size by the factors given by WRIGHT and EATON, 1929) show some tendency to increase with increasing age of the mother but the trends are slight and hardly significant.

The females do not reach full size until 15 months of age. On the whole it appears that the offspring from immature females are at some disadvantage, but this is very slight. The point established by these comparisons is that immaturity of the mother has a much greater influence on the development of an atavistic little toe by the young than on a number of characters, in which an effect would seem more likely on *a priori* grounds.

CORRECTIONS FOR AGE OF DAM

The important effect of age of dam makes it necessary to test whether other apparent effects may not be merely consequences of an irregular age distribution.

TABLE 26

The results of different types of matings classified by age of dam. Beltsville.

DAM	SIRE	AGE OF DAM (BELTSVILLE)								TOTAL		
		3-6		6-9		9-15		15-		NUMBER	PERCENT	WEIGHTED
		NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT			
			4-T		4-T		4-T		4-T			
3-toe	3-toe	198	42.9	209	34.4	337	20.5	275	16.4	1019	26.6	25.9
3-toe	4-toe	39	66.7	55	34.5	53	35.8	38	26.3	185	40.0	38.0
4-toe	3-toe	36	50.0	37	32.4	66	25.8	131	9.9	270	22.2	26.3
4-toe	4-toe	76	72.4	89	59.6	155	42.6	182	19.2	502	41.6	43.8
Total		349	52.7	390	40.0	611	28.0	626	16.5	1976	31.1	

Table 26 shows that correction for age distribution in the Beltsville stock has little effect on the production of different types of mating (last two columns). There is some increase in the regularity of the results. In the Chicago stock on the other hand, it happened that matings of 4-toe \times 4-toe produced very few young in the last age classes. On weighting the per-

TABLE 27
The results of different types of matings, classified by age of dam. Chicago.

DAM	SIRE	AGE OF DAM (CHICAGO)								TOTAL		
		3-6		6-9		9-15		15-		NUMBER	PERCENT	WEIGHTED
		NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT			
										4-T	4-T	4-T
3-toe	3-toe	17	88.2	31	35.5	44	34.1	48	35.4	140	41.4	43.7
3-toe	4-toe	14	64.3	9	44.4	19	21.1	29	13.8	71	29.6	30.6
4-toe	3-toe	9	100.0	11	36.4	20	45.0	30	23.3	70	41.4	45.3
4-toe	4-toe	19	94.7	22	63.6	28	32.1	8	37.5	77	57.1	50.6
Total		59	86.4	73	45.2	111	33.3	115	27.0	358	42.5	
3-toe parent		57	84.2	82	36.6	127	33.9	155	29.0	421	39.4	41.2
4-toe parent		61	88.5	64	56.3	95	32.6	75	22.7	295	46.8	43.5

centage of polydactyls from each type of mating and age class by the total number from that age class, the evidence for heredity largely disappears. On combining males and females to increase the number it appears that 3-toed parents produced 41.2 percent polydactyls and 4-toed parents only slightly more, 43.5 percent, although there was some tendency to mate 3-toe with 3-toe and 4-toe with 4-toe. The tetrachoric correlation between parent and offspring from this data is only $+0.03$ instead of $+0.11$. This result, in conjunction with the reduced correlation between litter-mates and between right and left foot in the data lead to the conclusion that genetic variation is practically absent in this group descended from a single mating in the 22nd generation of brother-sister mating.

SEASONAL EFFECTS

The Beltsville records of family 35 were tabulated by month of birth. Table 28 shows the percentage of 4-toed young in each month.

TABLE 28
Percentage of polydactyls by months of birth. Beltsville.

MONTH OF BIRTH	NUMBER	PERCENT 4-T	MONTH	NUMBER	PERCENT 4-T
May	136	28.7	November	161	32.3
June	160	24.4	December	159	30.2
July	231	24.2	January	160	44.4
August	176	29.0	February	149	39.6
September	140	24.3	March	148	43.9
October	215	23.7	April	141	34.8
May-October	1058	25.5	November-April	918	37.5

There is an unquestionably significant cycle with respect to polydactyly. The χ^2 test (24 classes, 11 degrees of freedom, $\chi^2 = 48$) yields a probability of .000002 that the differences can be due to random sampling with no allowance for the grouping of signs. But in every month from May to October there were less than 30 percent polydactyls (average 25.5 percent) and in every one of the remaining 6 months there were more than 30 percent (average 37.5 percent). A difference in average is shown within each of the four groups (table 29) into which the family has been divided and also from dams of each age class (table 30). This seasonal cycle accounts for about 3.5 percent of the total variance.

TABLE 29
Percentage of polydactyls in two 6-months periods in each group of substrains. Beltsville.

MONTHS OF BIRTH	GROUPS OF SUBSTRAINS							
	GROUP I		GROUP II		GROUP III		GROUP IV	
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T
May-October	214	10.3	356	18.3	362	28.6	226	47.8
November- April	172	14.0	320	25.0	236	49.2	190	65.3

TABLE 30
Percentage of polydactyls in two 6-month periods at different ages of the dams. Beltsville.

MONTHS OF BIRTH	AGE OF DAM							
	3-6		6-9		9-15		15-45	
	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T	NUMBER	PERCENT 4-T
May-October	157	46.5	224	37.1	346	23.4	331	10.0
November- April	192	57.8	166	44.0	265	34.0	295	23.7

It is somewhat disconcerting therefore to find that in the Chicago data the cycle is reversed. There were 51.5 percent polydactyls born from May to October and only 34.6 percent from November to April. This difference is 3.2 times its standard error on the basis of the numbers of young in the two periods, but becomes somewhat doubtful on allowing for the intra-litter correlation of +.54. This result can not, of course, invalidate the significance of the Beltsville cycle but makes it clear that it is not season *per se* which is responsible.

Table 31 shows the averages of a number of characters in the two 6-month periods which are most significant for polydactyly. A tabulation by

TABLE 31

Averages of various characters in two 6-month periods. Beltsville. Birthweight is of liveborn young.

MONTHS OF BIRTH	NUMBER	PERCENT 4-T	SIZE OF LITTER	PERCENT		BIRTH WEIGHT	33 DAY WEIGHT	PERCENT WHITE	
				BORN DEAD	DIED OF LIVEBORN			♂	♀
May-									
October	1058	25.5	2.50	21.1	17.8	80.7	229.8	61.1	68.5
November-	918	37.5	2.38	23.0	24.4	81.5	222.5	61.3	67.0
April									
Total	1976	31.1	2.44	22.0	20.9	81.1	226.6	61.2	67.8

months indicates that this division of the year is as significant as any in the other cases. It is remarkable that polydactyly should show a more marked seasonal variation (in the Beltsville data) than such characters as birth weight and percentage of stillborn (neither of which showed any appreciable cycle during this period) or size of litter, percentage of mortality between birth and weaning and weight at weaning. However, the litters were smaller, the postnatal mortality higher and the weaning weight lower during the period (November to April) in which the percentage of polydactyls was high, as compared with the remaining 6 months. On the whole polydactyly appears to be associated with unfavorable conditions. The percentage of white in the coat is included in table 31 as a character which agreed with polydactyly in showing an important effect of age of dam, but it shows no seasonal cycle in the same animals in which polydactyly shows a marked cycle. Even size of litter and the mortality percentages give no indication of a cycle in the Chicago data in which the cycle for polydactyly seems to have been reversed.

The most important difference between the Beltsville and Chicago conditions was probably in the green feed. There was a rather definite feeding cycle at Beltsville: freshly cut grass and green alfalfa from late spring to mid-fall, the period in which relatively few polydactyls were born; stored cabbage and kale, often inadequate in quantity or quality during the rest of the year when many polydactyls were born. Stored cabbage has been used throughout the year at Chicago.

About all that can be said is that some environmental factor, generally unfavorable, but with a relatively specific tendency to induce polydactyly, varied seasonally under the conditions at one laboratory, but is not inevitably associated with season as shown by results in another laboratory.

SIZE OF LITTER

Table 32 shows the percentages of 4-toed young in each size of litter. There are rather striking differences in the Beltsville data and ones which appear to be statistically significant even if litters instead of individuals

TABLE 32

Percentage of polydactyls among young born in each size of litter. Beltsville and Chicago.

SIZE OF LITTER	BELTSVILLE		CHICAGO	
	NUMBER YOUNG	PERCENT 4-T	NUMBER YOUNG	PERCENT 4-T
1	150	22.7	29	37.9
2	562	34.5	94	34.0
3	795	34.8	152	46.7
4	369	26.3	68	41.2
5, 6	100	12.0	15	66.7
Total	1976	31.1	358	42.5

are made the units. It is, however, difficult to interpret a high percentage in litters of 2 and 3 and low percentages in litters of 1, 4 and 5. The Chicago data do not confirm the order in the Beltsville data. About all that can be said at present seems to be that there are relations of some sort between factors which affect size of litter and ones which affect number of digits.

COMPARISONS OF 3-TOED AND 4-TOED INDIVIDUALS

There is not much difference in the characteristics of 3-toed and 4-toed individuals apart from digit number. The Beltsville data show a lower percentage of 4-toed born dead than of 3-toed (15.8 percent *vs.* 22.9 percent). Not much confidence can be placed in this, however, since it is possible that a rudimentary little toe was overlooked more frequently in the young born dead than in those born alive in these records in which digit numbers were recorded merely as a matter of routine in a large colony of which family 35 constituted only a small portion. In the Chicago data the feet were examined with the greatest care in all stillborn animals of family 35 and no significant relation was found (30.3 percent born dead among polydactyls and 33.0 percent among normals).

The percentage of liveborn which died between birth and weaning was higher among polydactyls in the Beltsville data (34.2 percent *vs.* 19.2 percent). There is no reason for any systematic error in the routine records here, since these animals were all alive when recorded. There is an indication here that the factors which determine polydactyly are otherwise unfavorable, confirming previous indications. The parallelism in the seasonal cycles of polydactyly and this mortality percentage of course contributes a little (about 0.9 percent) to the above difference. The Chicago data show no important difference between 4-toed and 3-toed in this respect (22.6 percent of the 4-toed and 21.7 percent of the 3-toed died before weaning).

The average birth weight of the 4-toed young born alive (81.2 grams) was practically the same as for 3-toed young (81.0 grams). The 4-toed,

however, had the advantage of being born in slightly smaller litters (average 2.72) than 3-toed (2.84). Correction for the regression of -11.3 grams per unit change in litter size brings the birth weight of 4-toed below that of 3-toed but hardly to a significant extent (difference 1.1 grams).

The average weights at weaning show more difference: 4-toed 221.4 grams, 3-toed 228.8 grams. The difference is increased to about 10.0 grams on allowing for the regression of -26.2 grams per unit change in litter size. This appears to be statistically significant, having a standard error of about 2.4 grams on the basis of a standard deviation of 38.5 grams (corrected for size of litter) and the number of animals involved (848 3-toed, 392 4-toed). It is significant even on increasing some 25 percent to allow for the intra-litter correlations of the characters involved. The parallelism in seasonal cycle contributes very little to this difference. Again there is an indication that the factors of polydactyly are on the whole of an injurious sort. More important, however, is the conclusion that the physical differences between 3-toed and 4-toed representatives of family 35 are of a trivial sort. The environmental factors determining polydactyly must be for the most part rather specific for this character.

SUMMARY

The recurrence of an atavistic little toe is not uncommon in strains of guinea pigs. Among 23 inbred families, 12 never produced 4-toed young, 5 produced a few sporadics, while the remaining 6 produced percentages ranging from 2 to 19 (1906 to 1915). A branch of one of these, derived from a single mating in the 12th generation of brother-sister mating produced 31 percent at Beltsville, Maryland. Subdivisions of this ranged from 9 percent to 69 percent. A large random-bred group derived from a single mating of this family in the 22nd generation, produced 42 percent (in Chicago).

It is assumed that the presence or absence of the little toe depends on whether the combination of factors exceeds or falls below a threshold. On a scale of uniform factor effects, 18 percent of the variance of the Beltsville stock was genetic, due to substrain differences (squared biserial η .18, also parent-offspring correlation .18). There was no demonstrable genetic variability within substrains. Genetic variability was also negligible in the Chicago stock (parent-offspring correlation .03, after correction for other factors).

Litter-mates were determined 62 percent by common factors in the Beltsville stock (correlation $+.62$). This includes the genetic factors and 54 percent of the non-genetic ones. In exact agreement is the correlation of .54 between litter-mates in the Chicago stock.

The non-genetic factors common to litter-mates are equally divided be-

tween ones also common to non-litter-mates from the same mating (correlation .27) and ones common only to litter-mates (Chicago stock). These results lead to the following analysis of variability.

	BELTSVILLE	CHICAGO
Hereditary differences (substrains)	18	0
Environmental differences common to		
Sibships	22	27
Litters	22	27
Individual	38	46
Total	100	100

There is frequent asymmetry. The correlation between left and right was .78 in the Beltsville data and .73 in the Chicago data. That between single feet of litter-mates was .59 in the Chicago data. These results in combination with the preceding lead to the following analyses of the factors responsible for digit numbers on a single foot.

	BELTSVILLE	CHICAGO
Hereditary differences	19	0
Environmental differences common to		
Sibships	24	29.5
Litters	24	29.5
Individuals	11	14
Foot	22	27
Total	100	100

Part of the variance assigned to the individual in the analysis of individual variability is spurious due to non-additive effects. The analysis of the variance of single feet is more satisfactory.

No direct evidence was obtained of the nature of the non-genetic factors affecting whole sibships. Presumably long-continuing conditions of the mother are involved.

The most important factor affecting whole litters is age of the mother. This determined 13 percent of the total variance in the Beltsville stock and (less reliably) 25 percent in the Chicago stock. Immature females produce much higher percentages of 4-toed young than mature females. The effect is primarily neither one of parity nor of weight of mother. Indeed the heavier females of a given age produce slightly more polydactyls than the lighter ones. A physiological competition for some substance between growing mother and embryo seems the most plausible suggestion.

A seasonal cycle, high percentage of polydactyls in late fall, winter and early spring, low the rest of the year, accounted for 3 percent of the total variance in the Beltsville stock. That this was not an effect of season *per se* was indicated by a reversal in the Chicago stock. The seasonal effect and relation to rate of growth and death rate, indicate that unfavorable conditions tend to increase the percentage of polydactyls. The 4-toed individuals are not, however, at all handicapped in prenatal growth or chances of live birth and only slightly in postnatal growth and viability.

No direct evidence has been obtained of the nature of the individual factors. The extremely local action of important factors is indicated by the frequent asymmetry. In this the left foot is slightly more likely than the right to develop a little toe. There is no relation to sex.

A recognition and evaluation of the importance of non-genetic factors in determining the presence or absence of the little toe is a necessary foundation for analysis of the genetic differences between different stocks.

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