HARELIP IN THE HOUSE MOUSE

I. EFFECTS OF THE EXTERNAL AND INTERNAL ENVIRONMENTS

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INTRODUCTION

THE character "harelip and cleft palate" is definitely inherited (REED and SNELL, 1931). Though there are various degrees of expression of harelip and cleft palate, the general condition, regardless of the types of clefts, will henceforth be designated simply as "harelip." Harelip is not inherited as a simple dominant or a simple recessive but seems to depend upon either one recessive gene with modifiers or the co-operation of a small number of cumulative genes. If the first hypothesis is correct, it must be assumed that when the recessive gene for harelip is homozygous, the mouse may show any grade of harelip from the most extensive bilateral clefts to a normal mouth without clefts, depending upon whatever genetic and environmental modifiers had an effect upon the embryo. The phenotypically normal, but genetically harelip, animals will be designated "normal overlaps."

It is usually easy to determine at birth whether a mouse is phenotypically harelip. Occasionally the cleft or clefts are so slight that they are hardly noticeable. Probably in several cases there have been slight lip and palate defects in genetically harelip animals which were not noticed. Such animals would have been erroneously classified as "normal."

Environmental influences greater than ordinary may be suspected in connection with variable characters such as harelip. These effects may be detected if found to occur in correlation with differences in litter size, weight, age of mother, and the like.

EXPRESSION OF HARELIP AFFECTED BY THE EXTERNAL ENVIRONMENT

One can not hope to measure these except with inbred stocks. Following the discovery of harelip in 1930, the stocks have been intensively inbred. At present there are three highly inbred lines and their substrains, all descended from the original harelip albino stock without outcrossing except in the early generations. For the last 10–14 generations a few matings have been made between daughter and father but nearly all have been between sister and brother.

The extent to which these three lines have been inbred and the variation in the percentages of harelip produced as inbreeding proceeded are shown in figure 1. Only the main branches of any of the lines are shown; the less important branches are usually of short duration before becoming extinct for one reason or another. The percentages of harelip in line 1 have increased as inbreeding continued whereas in line 3 they have decreased (down to 0 percent). Each point of each line in figure 1 was determined by the average percentage of harelip in 2–10 litters of the generation plotted. Line 1 has at this time reached a coefficient of inbreeding (Wright's) of over 95 percent; this is equivalent to some fourteen generations of brother

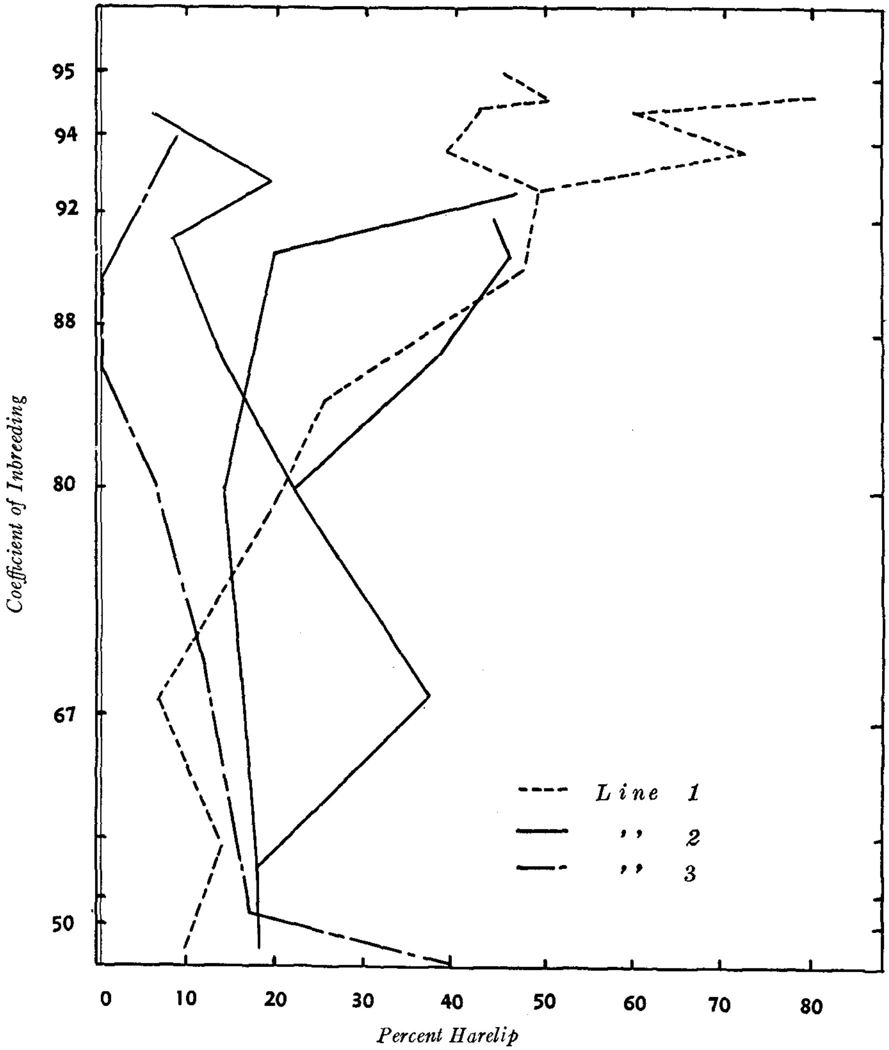

Figure 1. The main branches of three highly inbred harelip lines studied in this paper.

by sister inbreeding. We may assume that there is now practically complete homozygosity in each of these lines.

The difference in percentage of harelip between lines 1 and 3, in the last generations at least, is sufficiently great and consistent to indicate real genetic differentiation. In the following paper an attempt will be made to determine whether this difference is due to different combinations of the genes modifying one recessive essential for any harelip production or whether harelip depends upon the cumulative action of several genes of similar value, different groups of which produce characteristic frequencies of harelip.

Our first problem, however, is to test whether some of the variability within any one strain is due to environmental factors. The data for the study of environmental factors are derived from lines 1, 2, and 3 and their sublines (1a, 2a, 2b, etc.). In investigations such as that of the relation between sex and harelip expression, crossbred animals have been included with the inbred animals of lines 1, 2 and 3 but all cases where animals are included that have not been inbred for several generations are specified.

Litter size

It was shown many years ago that litter size is probably the most important single factor in determining the birth weight of guinea pigs, and that the larger the litter, the shorter the gestation period (MINOT 1891, WRIGHT 1921 and 1922, and EATON 1932). Probably the situation is similar in mice; GATES (1925) reports that in mice the birth weights tend to vary inversely with the litter size, while KING (1915) has shown that weight of rats at birth is in direct proportion to the length of the gestation period. Litter size accordingly would seem to be a factor worth investigating in its relation to harelip.

If there is a competition between embryos for some substance particularly concerned with normal development of the jaw and palate, supplied by the mother only in a certain quantity, the smaller the litter the more of the substance for each embryo. Accordingly, small litters should have a lower percentage of harelip than large ones.

Litter size was investigated in 525 litters. The first and second litters of each of the mothers are included and as many following litters in consecutive order as it was possible to include. Each of the litters was observed within 24 hours of birth. All the litters from any one mother had the same father. All the parents were from the inbred stocks (lines 1, 2 and 3) derived in 1930 from Bagg albinos.

Many precautions have been taken in selecting proper litters for the study of environmental effects, with the intention that all material might be strictly comparable. In the statistical treatment, the standard error has been used in preference to the probable error.

TABLE I

Distribution of harelip litters by size and by number of harelip (hp=harelip animals)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS WITH						TOTAL LITTERS	EXPECTED NO. OF LITTERS WITH						
		0hp	1hp	2hp	3hp	4hp	5hp		0hp	1hp	2hp	3hp	4hp	5hp	6hp
A q=.1059	1	3						3	2.7	0.3					
	2	10	3					13	10.4	2.5	0.1				
	3	24	8	1				33	21.1	10.1	1.7	0.1			
	4	32	6	1				39	24.9	11.8	2.1	0.2			
	5	25	21	4				50	28.6	16.9	4.0	0.5			
	6	33	19	4	4			60	30.7	21.8	6.4	1.0	0.1		
	7	27	20	9	2			58	26.5	22.0	7.8	1.5	0.2		
	8	15	10	6	5			36	14.7	13.8	5.8	1.5	0.2		
	9	10	4	3				17	6.2	6.7	3.2	0.9			
	10		2	7				9	3.2	3.5	1.9	0.6			
	11		1					1	.3	.4	0.3	0.0			
	12	1	2					3	.8	1.1	0.8	0.3			
Total		180	96	35	11			322	169.9	110.9	34.1	6.6	.5		
B q=.2889	1	2	1					3	2.1	0.9					
	2	5	3	1				9	4.6	3.7	0.7				
	3	4	2	3	1			10	3.6	4.6	1.8				
	4	8	4	3	1			16	4.1	6.7	4.1	1.1			
	5	8	5	9	4	2		28	5.1	10.4	8.4	3.4	0.7		
	6	6	6	6	6		1	25	3.2	7.9	8.0	4.4	1.3	0.2	
	7	4	4	13	5	3		29	2.7	7.6	9.2	6.3	2.5	0.6	0.1
	8	1	2	4	3	2	3	15	1.0	3.2	4.5	3.7	1.9	0.6	0.1
	9			2		1		3	0.2	0.5	0.9	0.8	0.5	0.1	0.0
	10		1	2	1	2		6	0.2	0.9	1.5	1.6	1.1	0.6	0.1
Total		38	28	43	21	10	4	144	26.8	46.4	39.1	21.3	8.0	2.1	0.3

TABLE I (Continued)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS										TOTAL LITTERS
		0hp	1hp	2hp	3hp	4hp	5hp	6hp	7hp	8hp	9hp	
	1	1	1									2
	2	1	1									2
	3	3	1	3								7
	4	3	2	3	2							10
C	5		1	1	5	2						9
q=.4859	6		1	3	6	2						12
	7				6	1	1	1				9
	8					3		1				4
	9						1	1		1		3
	10											
	11										1	1
Total		8	7	10	19	8	2	3		1	1	59
		EXPECTED NO. OF LITTERS										
	1	1.0	1.0									2.0
	2	0.5	1.0	0.5								2.0
	3	0.9	2.7	2.6	0.8							7.0
C	4	0.7	2.6	3.7	2.4	0.6						10.0
	5	0.4	1.5	2.9	2.7	1.3	0.2					9.0
q=.4859	6	0.2	1.3	3.0	3.8	2.7	1.0					12.0
	7	0.1	0.6	1.6	2.5	2.4	1.4	0.4				9.0
	8	0.0	0.2	0.5	0.9	1.1	0.8	0.4	0.1			4.0
	9		0.1	0.3	0.5	0.8	0.7	0.4	0.2	0.0		3.0
	10											
	11		0.0	0.0	0.1	0.2	0.2	0.2	0.2	0.1	0.0	1.0
Total		3.8	11.0	15.1	13.7	9.1	4.3	1.4	0.5	0.1	0.0	59.0
GRAND TOTALS A–C												
Observed		226	131	88	51	18	6	3		1	1	525
Expected		200.5	168.3	88.3	41.6	17.6	6.4	1.7	0.5	0.1	0.0	525.0

In the quantitative determinations of the amounts of variance affected by various influences such as litter size a smaller population has been used. This is composed of animals from line 1 and its recent branch 1a. This population of 1284 animals will be referred to as the "small population," and will be used for all biserial eta and tetrachoric correlations.

Most environmental factors would act on litter mates alike. Malnutrition, disease and age of mother, if effective, would tend to make litters have excesses of either harelip or normal as the conditions might determine. A method of ascertaining whether or not there are disturbances in the distribution of normal and abnormal animals among the litters has been developed by WRIGHT (1934a) and will be used here. It is possible to calculate the expected occurrence of harelip in litter mates as well as the number of harelip animals expected for each size of litter.

Each mother may be placed in one of three groups in respect to the percentage of harelip young produced in all her litters. In table 1, Group A includes all the litters from mothers which produced up to 21 percent of harelip young (as an average of the fraternity). Group B includes all the litters from mothers which produced from 21 to 40 percent of harelip young. Group C includes all the litters from mothers producing from 41 to 60 percent of harelip young.

The distribution of harelip in litters of each size, expected under random sampling, can be calculated as follows. Let q be the chance of abnormal development in the group in question, and $(1-q)$ the chance of normal development. In litters of 2 the chance that both will be normal is $(1-q)^2$; that one will be harelip and one normal is $2q(1-q)$; and finally that both will be harelip is q^2. In litters of 3 the chances of 0, 1, 2, 3, harelip are the appropriate terms in the expansion $[(1-q)a+qA]^3$ where a stands for normal and A for abnormal. The expectations for larger litters are given by expansion of the appropriate power of the binomial.

The degree of agreement of the grand totals of table 1 is shown in table 2. Note that there were many more litters observed than expected in which there were no harelip offspring and a considerable deficiency of litters containing one harelip animal.

TABLE 2

Relation of the observed number of litters containing 0–9 harelip offspring to the number expected. (hp = harelip)

	0 hp	1 hp	2 hp	3 hp	4 hp	5–9 hp	Total
Observed $(m+x)$	226	131	88	51	18	11	525
Expected (m)	200.5	168.3	88.3	41.6	17.6	8.7	525.0
x^2/m	3.24	8.27	0.00	2.12	0.01	0.61	14.25

There are six classes in the table and two degrees of freedom are lost—one by accepting the total number and one (approximately) by accepting the proportion of harelip in each group. With four degrees of freedom the chance that such deviations would occur from random sampling is less than 0.01 and because of this small probability, the deviations are undoubtedly significant. The deviations are of the order expected if there were a tendency for members of a litter to be similarly affected by environmental factors. Among other factors, age of mother, if significant, could have caused deviations of this order.

Table 3a presents the data for the large population (lines 1, 2 and 3). It will be noted that small litters are deficient in harelip, litters of 5–7 agree with expectation while there is an excess of harelip in large litters. The χ^2 test shows that the correlation between large litters and higher percentage of harelip is undoubtedly significant; total $\chi^2 = 16.04$ with only two degrees of freedom.

TABLE 3a

Significance of the relation between size of litter and number of harelip in the litter (whole population)

Litter size	1–4	5–7	8–12	Total
Observed hp (19.6%) (m+x)	74	345	178	
Expected hp m	103.3	339.3	154.4	
Deviation x	−29.3	+5.7	+23.6	
x^2/m	8.30	0.96	3.62	12.88
$\chi^2=\left(1+\frac{.196}{.804}\right)\Sigma\frac{\chi^2}{m}$				
$\chi^2=1.244\times12.88$ Total $\chi^2=16.04$				

TABLE 3b

Significance of the relation between size of litter and number of harelip in the litter (small population)

Litter size	1–4	5–7	8–12	Total
Observed hp (25.1%) (m+x)	48	179	100	327
Expected hp m	55.3	184.6	87.1	327.0
Deviation x	−7.3	−5.6	+12.9	
x^2/m	0.96	0.17	1.91	3.04
$\chi^2=\left(1+\frac{.251}{.749}\right)\Sigma\frac{\chi^2}{m}$				
$\chi^2=1.335\times3.04$. Total $\chi^2=4.06$				

In the small population the correlation is of the same type as that for the large population although χ^2 is not significant. There can be no doubt, however, that the effect of size of litter is present in the small population even though not in such a pronounced degree (table 3b).

The actual correlation was calculated for the small population (1284 animals). Biserial eta squared is equal to 0.035. This means that 3.5 percent of the total variation in harelip production of the small population is due to the litter size. WRIGHT'S formula for biserial eta squared is, eta squared $=\sigma^2/1+\sigma^2$. The sigma is that for the total group of arrays.

Age of the mother

King (1917) and others have found a relationship in rats between age of mother and birth weight of young. In all cases the young from mature dams were heavier than those from young females. If the age of the mother is an important factor in the expression of harelip, young mothers might be expected to produce a higher percentage of harelip young than mature ones.

When the mean percentage of harelip produced by mothers of the various ages is calculated, we find larger fluctuations than we should expect from chance alone. The percentage of harelip young is high in the litters of 2 and 4 months old mothers but, by exception, is very low in the case of mothers 3 months old. Subjected to all manner of tests, this low percentage from 3 months old mothers remains. It is always consistent and significant. The percentage for mothers 4–7 months of age is about that found for 2 months old mothers, but for mothers 8–10 months old, the percentage is very low (table 4).

It was thought necessary to study those mothers which were still producing litters at 7–10 months of age. By selecting only those mothers which produced offspring from the time they were about 2 months of age until they were about 7–10 months old we avoid fluctuations in the data resulting from differences in harelip frequency of different fraternities of young. As an illustration, if one mother produced an average of 50 percent of harelip progeny in all her litters but produced none after she was 6 months old, while a second mother averaged only 25 percent harelip, but produced them through 10 months of age, there would be a spurious drop in harelip production after 6 months of age observed in the combined data of the two females. Mothers which are more nearly homozygous for genes influencing harelip might produce higher percentages of harelip but fail to produce young after 6 months of age. Therefore the following calculations are from mothers which produced about one litter a month from the age of 2 months to 10 months.

The lower part of table 4 shows the significant drop in harelip production at 3 months of age. This drop, which is the only statistically significant fluctuation, is impossible to explain physiologically at present. The drop in harelip percentage comes suddenly with mothers 10 weeks of age, while the abrupt rise begins at about 15 weeks. Examination of several groups of females separately shows, consistently, the drop in percentage of harelip produced by 3 months old mothers.

A χ^2 table was made to test whether there is an association between harelip expression and age of mother. In this table there were eight age groups against the two alternatives harelip and not-harelip, seven degrees of freedom, and a total χ^2 of 18.01. It is probable that such a large χ^2 would result from chance factors alone only about once in one hundred times. Therefore there is a significant relationship between the expression of harelip and the age of the mother.

TABLE 4

The percentages of harelip young born to mothers from 2–11 months old

AGE OF MOTHER (MONTHS)	NO. OF LITTERS	HARELIP YOUNG (PERCENT)
2	45	30.9±3.5
3	83	19.6±2.2
4	85	25.2±2.4
5	55	30.9±3.4
6	38	23.5±3.5
7	23	22.0±4.1
8	17	17.2±4.3
9	10	16.1±4.7
10	3	11.3
11	2	0.0

Data from mothers still producing at 7–10 months

AGE OF MOTHER (MONTHS)	NO. LITTERS	HARELIP YOUNG (PERCENT)	DIFFERENCE
2	52	29.9±3.2	
			2.2^{σ}
3	100	21.2±2.2	
			2.6^{σ}
4–5	172	28.8±2.0	
			0.7^{σ}
6–7	95	31.5±3.1	
			1.8^{σ}
8–10	56	23.3±3.5	

As a descriptive statistic it would be interesting to know how great the correlation is between age of mother and the expression of harelip. The use of the coefficient of correlation (r) is valid only when the regression is rectilinear; therefore it is better to use the correlation ratio (eta), which is suitable for both rectilinear and curvilinear distributions. Biserial eta was used in this case where the two alternatives, harelip and not-harelip, are correlated with the different ages of mother. Biserial eta was found to be 0.164. The error of this correlation is not included, the χ^2 test having shown the relation between expression of harelip and age of mother to be significant. The symbolism and formulae employed in determining eta were taken directly from WRIGHT (1934b, p. 513–514).

We have found eta = 0.164. Eta squared is 0.027, indicating about 3 percent determination of the total variance of harelip by the age of mother. Considering the inexplicable drop in harelip production of 3 months old mothers, this finding of 3 percent variance has not been considered in our final analysis; it is merely indicated that age of mother is a factor, but its quantitative determination is left in abeyance.

Wright's (1934b) valuable paper on polydactyly shows "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 (such as mortality at birth) in which an effect would seem more likely on *a priori* grounds." I assume that the age effect in the case of polydactyly may parallel the age effect on harelip. I assume that if there is an effect of age, it is a direct physiological one acting upon the early embryo.

Time interval between litters

The gestation and nursing periods in mice each take about three weeks. Females often carry a litter while nursing the litter recently born and may, for a period of several months, both carry and nurse successive litters at the same time, weaning one litter at the birth of the next one. Such a reproductive load could conceivably affect expression of harelip. We may investigate this possibility by use of the time interval between successive litters. If a litter is born 3 or 4 weeks after the birth of the previous litter, it is likely that this litter was carried while the previous litter was nursing. If, however, the litter is born 6 weeks after the previous one, this previous litter will have been weaned before gestation of the later one. We may compare the percentages of harelip contained in litters born 3, 4, 5, or 6 or more, weeks after the birth of the previous litter. Percentages of harelip for each litter were not calculated, but the mean percentage of harelip of all litters born at the specified period. The material is all from the small population.

NO. OF LITTERS	NO. OF WEEKS ELAPSED SINCE BIRTH OF THE PREVIOUS LITTER	PERCENT HARELIP
89	3	35.0
25	4	39.8
12	5	44.5
35	6 or more	30.6

We may well combine the data for 3 and 4 weeks (114 litters with 36.0 percent harelip) and for 5 and 6 weeks (47 litters with 34.4 percent harelip). The difference between these percentages could easily be due to chance alone. There is, then, no significant effect of the length of the time interval between litters upon the expression of harelip.

A further related attempt was made to analyze effects of the condition of the mother on the expression of harelip in the young. One might expect to find an association between the nursing of the previous litter and a

TABLE 6

Harelip (H) in relation to age of mother (A) and birth rank (B)

BIRTH RANK	AGE OF MOTHER IN MONTHS																	
	2		3		4		5		6		7		8		9–10		TOTAL	
	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP
1	81	30.9	100	17.0	36	33.3											217	24.9
2			70	21.4	101	28.7	56	21.4									227	24.7
3					66	42.5	115	27.8	64	26.6	4	25.0					249	31.3
4							41	34.2	15	25.2	63	28.6	7	28.6			262	27.5
5									47	29.8	61	16.4	48	18.8	12	33.3	168	22.0
6											16	25.0	64	17.2	22	27.3	102	20.8
7													3	0.0	44	18.2	47	17.0
8															12	16.7	12	16.7
Total	81	30.9	170	18.8	203	34.0	212	27.4	262	26.3	144	22.9	122	18.2	90	22.2	1284	25.6

higher percentage of harelip in the next litter, if this next litter were *in utero* while the previous litter was nursing. This was not the case. If the previous litter was nursed, 27 of the litters which followed had a higher percentage of harelip than that of each corresponding previous litter; the remainder of the litters that followed (38) had a lower percentage of harelip than each of the corresponding previous litters. If the previous litter was removed at birth and not nursed, 63 litters arriving within the next month contained a higher percentage of harelip than the corresponding previous one, and 62 contained a lower percentage.

Lactation has no apparent influence upon harelip expression.

Birth rank

The correlation between expression of harelip and age of mother ($\eta_{HA} = -.16$) allows us to be fairly certain that a correlation between birth rank and expression of harelip will be found because age of mother and birth rank bear an obvious relationship to each other. The correlation between birth rank and expression of harelip proved to be $\eta_{HB} = -.12$. This is not as great as the correlation between age of mother and harelip expression and indicates that age of mother and not birth rank is a factor involved in the variation of harelip expression. Table 6 presents the data concerned with both birth rank and age of mother.

The correlation between age of mother and birth rank is of course high ($r_{AB} = +.96$). It is concluded that birth rank has no effect on the expression of harelip.

Seasonal variation

It would be reasonable to assume that a character such as harelip might be influenced in its expression by seasonal changes. The temperature of the mouse room was held fairly near 70°F during the winter months, but through late spring, summer, and autumn there were considerable fluctuations due to the effect of heat from outside. In spite of such temperature and seasonal changes there seems to have been no significant variation in the percentage of harelip young (table 7).

Though there seem to be no seasonal differences of a regular sequence in harelip production it might be well to investigate periods of a shorter duration. Some care was exercised in studying day-to-day periods but no significant fluctuations were found in the proportions of harelip offspring produced in the shorter periods.

Feed

The only feed used was a balanced ration sold as a fox chow. Fresh water was always present. As no vegetables were given, the feeding may be considered a constant factor which would have an equal effect, if any, on all the matings.

Uterine resorption

It is conceivable that harelip embryos are absorbed *in utero* in cases where the condition might be serious enough to be lethal at early stages. Line 2 has been so derived that all animals of the last few generations are over 90 percent inbred. We find that this line is producing about 15 percent harelip in these generations, and that the average litter size is 6.8 (32 litters). This is a large litter size for mice inbred to such an extent and in which there has been no selection for litter size. It is probable that there could have been but a very small prenatal death rate of harelip zygotes after implantation in this line at least.

TABLE 7

Absence of relationship between the season of the year and the percentage of harelip

SEASON	NO. OF LITTERS	PERCENT HARELIP
December–February	75	23.3±2.6
March–May	142	24.3±2.0
June–August	122	22.0±1.8
September–November	30	30.5±4.1
December–May	217	23.9±1.6
June–November	152	23.6±1.7
March–August	264	23.2±1.4
September–February	105	25.3±2.2

The difference between the incidence of harelip in line 2 (15 percent) and in line 1 (50 percent), with the litter sizes as they are, could be explained only on some assumption other than that of a difference in resorption. A study of the embryology of harelip (REED, 1933) revealed no evidence of differential prenatal resorption of the extreme cases of harelip.

It seems improbable to the writer that there is early zygotic elimination of harelip animals. If there is zygotic elimination it may be discovered in the future if a close linkage of harelip with some "regular" character is found.

We have now investigated, within the limits of our data, the important agencies of the external environment which might influence the expression of the character. We have found no discernible effect of seasonal fluctuations, feed, birth rank, or uterine resorption. There is evidence that the condition of the mother, as measured by age, has some effect and that litter size has an effect of 3.5 percent on the total variance of harelip.

EXPRESSION OF HARELIP MODIFIED BY INTERNAL ENVIRONMENT

There were 548 males to 460 females with some type of cleft, and 272 clefts of the left side alone and 218 of the right side alone in all the harelip populations. The difference in each of the comparisons (sex and symmetry) is statistically significant.

Sex

Taking all the litters in the harelip stock in which all members were sexed at birth (undepleted litters), we find a total of 735 males to 754 females, including both harelip and normal animals. We have noticed a marked excess of males among the harelip animals, but a slight excess of females when all animals are considered; it follows that among the non-harelip there must be an excess of females. There were 412 normal females to 320 normal males. Most of these excess females are probably normal overlaps for harelip.

In human races the fact is unquestionable that harelip occurs with greater frequency in males than in females, and on the left side than on the right. This agrees with the observations on mice. In man the most serious cases are significantly more common among males than among females (SANDERS, loc. cit.) and this is perhaps true in mice. When all grades of clefts are grouped, there are 54.4 percent males; but if only the severe cases are considered there are 55.5 percent males. Further, there is an eye defect associated with the harelip in my stocks which is perhaps another type of expression of the character. It agrees in showing an excess of affected individuals of the male sex. Of the total of 203 animals with eye defects, 68 were sexed; 51 were males and only 17 were females.

The association of harelip and these eye defects is statistically significant. In the litters in which animals with eye defects appeared, there were 60 animals with both eye defects and harelip, 143 with eye defects but no harelip, 150 with no eye defects but with harelip, and 616 animals without either eye defects or harelip ($p_1 - p_2$ is equal to $.100 \pm .032$ where p_1 = percentage of eye defects in harelip animals). The eye defect is similar to that found by other workers in other strains of mice and appeared in the harelip strains. Its relation to defects reported by other workers is not known. One or both eyelids may be open at birth, often followed by considerable damage to the adult eye.

In man the usual interpretation of the sex and symmetry differences is that males are weaker before birth and that the left side develops more slowly than the right (SANDERS, 1934); therefore the excess of male and left side harelip. As we have no reason to assume that more males than females have the genetic basis for harelip, we may suggest that owing to weaker development of the male, the genotype for harelip can express itself there more often than it can in females, where the threshold is not so readily exceeded.

Cyclopia in man and lower mammals appears more frequently in females than males (WRIGHT 1934a). The threshold here is crossed more easily in females than in males whereas with harelip the threshold is more easily exceeded in males. Presumably the two "thresholds" have quite different biological bases.

We may calculate the effect of sex on the total variance of harelip expression. In the small population (1284 animals) in which we determined the amount of variance due to age of mother, there were 166 harelip males, 476 non-harelip males, 135 harelip females and 507 non-harelip females. The harelip males constituted 25.8 percent of all the males and the harelip females 21.0 percent of all females in the small population.

We shall assume that there is a normal distribution of factor complexes underlying the dichotomy of harelip *versus* not-harelip on a scale in which the factors have additive effects. Then if σ is taken as the unit of measurement we may find the value of σ^2 after determining the means on our normal curve for males and for females. The mean of the males, and in like manner for the females, is found to be the value of the inverse probability integral of the percentage of males which are harelip minus one-half. Thus,

percent harelip (q)		$q-\frac{1}{2}$	$\text{prf}^{-1}\,(q-\frac{1}{2})$
♂♂	25.8	24.2	.650 = ♂ mean
♀♀	21.0	29.0	.806 = ♀ mean

Then,

$$\tfrac{1}{4}(\text{m}♀ - \text{m}♂)^2 = \sigma_{\text{sex}}^2$$
$$\tfrac{1}{4}(.806 - .650)^2 = .061$$

It will be recalled that the correlation *squared* between harelip and size of litter, for instance, is an indicator of the total effect of size of litter on harelip expression in the particular population which we have studied. For sex we found $\sigma^2 = .061$ so η^2 must equal 0.058. There is then a correlation, η^2, of nearly 6 percent between harelip expression and sex.

Asymmetry

The higher frequency of left clefts (55.5 percent) contrasted with right clefts (44.5 percent) brings up the problem of asymmetry.

With harelip a small portion of the asymmetry is inherited as there are significantly more clefts of the left side alone than of the right side alone. CASTLE (1906) found that a majority of his polydactylous guinea pigs were sinistral, but that there was no specificity in transmission, and his endeavor to increase the sinistrality by selection was unsuccessful.

Harelip in mice (and probably in man) behaves in just this way. From animals 90 percent inbred one can predict that, on the average, more left than right clefts will appear in their offspring, but it is found that there is no comprehensible order in the appearance of the left and right clefts. If there were particular genes for normal (or abnormal) development of the left or right side of the face, one would expect that, as inbreeding proceeds, there would be a gradual segregation so that if unilateral clefts continued to appear they would be more often on one side of the face in any inbred line. The most highly inbred lines (table 10) show no tendency for clefts of either the right or left sides alone to become established in any inbred line. The conclusion that the abnormality is inherited but that the asymmetry is not seems to be justified if we allow for the exception of the excess of left clefts.

TABLE 10

Absence of inheritance of left or right asymmetry (WRIGHT'S *coefficient of inbreeding*)

NO. OF ANIMALS IN FRATERNITY	COEFFICIENT OF INBREEDING	LEFT CLEFT	RIGHT CLEFT
LINE 1 (IN PART)			
6	.594	0	0
39	.672	2	0
13	.734	1	0
10	.785	0	1
36	.826	3	0
10	.859	1	0
24	.886	4	1
54	.908	1	0
60	.925	0	2
50	.940	0	2
42	.951	2	1
LINE 2 (IN PART)			
21	.785	2	0
55	.826	4	8
51	.859	3	0
85	.886	3	2
46	.908	3	2
102	.925	11	7
20	.940	0	0

There is another interesting observation on asymmetry. The eye defect associated with harelip showed an excess of affected males as did harelip itself; there is agreement as far as sex is concerned, but the asymmetry is exactly the opposite. There are more eye defects of the right side alone than of the left side, whereas with harelip the majority was on the left. There were 98 animals with the defects of the right eye, 60 with defects of the left eye and 45 with defects of both eyes.

WRIGHT'S (1934a) analysis of otocephaly shows no greater percentage of high grades of otocephaly among the more prevalent otocephalic females than among the fewer otocephalic males. With harelip we have seen that the percentage of higher grades is slightly greater (though not significantly so) among the males than among the females.

There is at least one distinction between the behavior of harelip and otocephaly. As the total frequency of harelip increases, the grade of the abnormality also increases; whereas with otocephaly, the grade of abnormality tends to decrease as the total frequency increases. In the common inbred harelip strains (high harelip frequency) 52.5 percent of the harelip animals were of the two highest grades (most severe cases), but of the various F_2 and double outcross harelip animals (low harelip frequency) only 31.5 percent were of the two highest grades. An arbitrary, but concrete, system of five grades was used.

With inbreeding the percentage of extreme cases of harelip appears to increase even though the absolute percentage of harelip of all grades may be dropping. In three harelip lines (2a, 2b, and 3) the percentage of harelip dropped with inbreeding, but the percentage of harelip animals of the two highest grades probably rises in an absolute sense (table 11).

TABLE 11

Data for lines 2a, 2b, and 3 of comparable generations

COEFFICIENT OF INBREEDING	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES
70±	22.3	40.6
80±	15.5	74.3
90±	10.5	58.3

In line 1a, in which the percentage of harelip has been rising, the percentage of cases which were of the two highest grades has also increased (table 12).

TABLE 12

Increase of frequency and severity of harelip in line 1a

COEFFICIENT OF INBREEDING (PERCENT HOMOZYGOSIS)	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES	NO. OF HARELIP
.734	7.7	0	1
.785	20.0	50	2
.826	26.3	60	5
.859	45.0	66	3
.886	46.0	55	11
.908	71.5	93	15
.925	59.3	100	16
.940	79.4	96	23

It might be expected that the fraternities of young among which there were the highest percentages of harelip would also show higher grades of harelip than fraternities with low percentages of harelip. This is not necessarily true because the most severe cases usually become predominant over the lower grades of harelip only after considerable inbreeding, whereas high percentages of harelip may or may not accompany extended inbreeding. It was found that the 280 harelip born in fraternities of less than 30 percent harelip were of the lower grades in 167 instances, and of the two highest grades in 113 instances. Of the 301 harelip animals born in fraternities of over 30 percent harelip, 149 were of the lower grades and 152 of the higher grades. From the fourfold table constructed with these data, we find that $\chi^2 = 5.3$; there is one degree of freedom. There is, therefore, probably a significant association between the two highest grades of harelip and the fraternities with over 30 percent harelip.

We have found that there is no tendency for the asymmetry (side affected) to become fixed with increased inbreeding. Late inbred generations still produce left and right clefts in about the same proportion as did earlier generations. With inbreeding the variability of the expression of harelip decreases; that is, with inbreeding there are fewer unilateral clefts and more bilateral (52.5 percent extreme cases in inbred stocks but only 31.5 percent extreme cases in crossbred stocks). Though the variability of harelip expression (severeness) always seems to decrease it is clear from tables 11 and 12 and figure 1 that as the inbreeding goes on the percentage of harelip may either increase or decrease. As inbreeding progresses the character is expressed more completely and severely irrespective of the percentage of harelip.

ALLOCATION OF THE RELATIVE INFLUENCES OF ENVIRONMENTAL AND GENETIC FACTORS

WRIGHT has developed methods for determining the relative influences of heredity and environment on the variation of characters similar to harelip. It is not easy to apply some of the methods to characters which are lethal and overlap as does harelip, but the following attempts have been made. I am deeply grateful to Professor WRIGHT for advice and assistance given while the paper was being written.

The correlation between parent and offspring could be used to determine the relative influence of heredity in regard to variation of the character. Unfortunately the parents do not have visible harelip, so it is impossible to make a direct parent-offspring correlation between individual parents and offspring. It is possible to determine in a qualitative way whether or not there is some correlation between parent and offspring by finding the ordinary correlation coefficient between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring.

The correlation between the percentage of harlip in the fraternity (large population) in which the mother was born and the percentage of harelip she gave was $r = +.333 \pm .093$ while that between the percentage of harelip in the fraternity in which the father was born and the percentage he sired was $r = +.186 \pm .104$. The average of these two correlations is $r = +.26 \pm .07$ which is fairly large and certainly significant. There is, then, a correlation between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring, though we can not use the above figures as a direct quantitative test of the amount of variance due to heredity.

What portion of the variability in our population of 1284 animals due to both heredity and environment is common to substrains? We know that the differences common to substrains, but not common to the whole small population, would be wholly genetic. It will be shown shortly that the variation common to sibships is equal to 10 percent. This *includes* the variation common to substrains, and this substrain variation should be equal to 10 percent or less.

The amount of variation common to substrains, but not to the whole population, can be calculated. The population of 1284 animals was divided into its substrains (the family tree partitioned into its branches or groups of fifty or more animals), the mean found for each substrain, $\mathrm{prf}^{-1}(q-1/2)$, and finally biserial eta squared for the total groups. Biserial eta squared was found to be 0.109; therefore about 11 percent of the total variability is common to substrains. The 10 percent variation, common to both substrains and sibships, theoretically should include this 11 percent common to substrains. The reason for the discrepancy is that there is apparently little, if any, variability common to sibships. Otherwise the agreement is as close as could be expected.

If variation were common to sibships, it would probably be divided between genetic differences of sibships and persistent conditions of the mother. Table 3 of the following paper contains evidence that there are probably no persistent conditions of the mother which affect harelip (age of mother, etc., are not persistent conditions). We may conclude that about 11 percent of the total variation in harelip expression is common to substrains and that little if any of the total is common to sibships alone.

The determination of the 10 percent of variation common to sibships and substrains was found by comparing consecutive litters of the sibships. It has just been noted that the variation is practically all common to substrains and none to sibships. The present determination of 10 percent is useful as a check on the previous finding of 11 percent for substrains. The comparison between each individual of an earlier litter and each individual in the next litter was carried out. If there were 2 harelip and 3 non-harelip in the earlier litter and 1 harelip and 4 non-harelip in the next litter, we could form a 2×2 table such as table 8. The actual data for our small population (1284 animals) are in table 9. It is necessary to use the tetrachloric correlation for a normal frequency surface (Kelley 1924). The correlation was found to be $r_t = +.100$ or 10 percent.

TABLE 8 TABLE 9

Correlations between individuals of different successive litters from the same mating

		ILLUSTRATIVE LATER LITTER				ACTUAL DATA LATER LITTER		
		NOT HARELIP	HARELIP	TOTALS		NOT HARELIP	HARELIP	TOTALS
Earlier Litter	Not harelip	12	3	15	Not harelip	3402	1097	4499
	Harelip	8	2	10	Harelip	1121	485	1606
	Totals	20	5	25	Totals	4523	1582	6105
							$r_t = +.100$	

The analysis of variation common to litters but not to sibships is now possible. If we compare individuals within each litter of the small population in a manner somewhat similar to that used in comparing litters of each sibship we find that the amount of the total variation common to litter mates, and including that common to substrains, is 21 percent. We know already that the variation common to substrains is 11 percent so we may subtract this from 21 percent and get 10 percent of the total variation which is common to litters but not to sibships or substrains. The variance common to litters is divided into 4 percent due to the effect of size of litter and 6 percent miscellaneous effects as yet unaccounted for.

The variation peculiar to the individual is a result in small part of its sex (6 percent) and of any segregation within litters. The amount of segregation should be slight as all members of each litter are certainly homozygous for the main harelip genes and probably for the same set of modifiers in each litter. Our residual, the environmental factors affecting the individual, accounts for nearly 75 percent of the total variation in harelip expression of this population which is approximately homozygous. It is interesting that such intangible factors as accidents of implantation should play such an important part in the non-genetic variation of an organism.

We may conclude that the small population studied quantitatively (1,284 animals) was practically homozygous and that the variation in the expression of harelip was due in small part to sex, litter size and age of mother, etc., but in the major portion to intangible chance factors working from within the mother but not correlated with her activities. Such intangible factors might be accidents of implantation, proximity of embryos, blood supply, etc.

Stated in another way, if all members of late generations of an inbred line possess the same genotype for harelip, whether the individual will be phenotypically harelip depends in small measure upon its sex, the litter size, the age of its mother, etc., while the main determining factors are those of accident or chance. We have no knowledge as yet just which chance factors are most effective.

Thus harelip is similar to white spotting in the guinea pig (WRIGHT 1920) in which, after homozygosis has been reached, further variation (which may be quite considerable) is due mainly to accidental influences affecting each individual more or less independently of his sibs. Such is the case with both white spotting and harelip. The similarity in behavior of harelip and of otocephaly in the guinea pig is even more pronounced.

SUMMARY

In mice there are differences in harelip expression resulting from the action of both environmental and genetic factors. Differences in harelip expression depend upon the sex of the individual, the size of the litter in which the individual was born, the age of its mother, asymmetry of the clefts, and in large part upon intangible chance factors. The variation in harelip expression resulting from these intangible chance factors may be best studied in populations in which all members are genetically harelip.

In a population of 1,284 mice from highly inbred families there was approximate homozygosis of genetic factors including those for harelip. However, non-harelip animals still appear and there is considerable variation among the animals which are phenotypically harelip. The allocation of the effects of the various factors controlling expression of harelip in these families is considered to be of about this order:

Variation common to substrains		11 percent
(but not common to the whole small population)		
Wholly genetic		
Variation common to sibships but not to substrains		0 percent
Genetic differences between sibships	little, or none	
Persistent conditions of the mother		
Variation common to litters but not to sibships		10 percent
Size of litter	4 percent	
Condition of mother at a given time		
Age of mother	?	
Season	0 percent	
Miscellaneous	6 percent	
Variation peculiar to the individual		79 percent
Sex	6 percent	
Individual genetic factors (slight)		
(segregation within litters)		

Factors of the environment (residual)	73 percent	
Possibly: Accidents of implantation Proximity of embryos Blood supply		
		100 percent

It is possible to simplify our presentation of the results in this fashion:

1. Variation due to hereditary differences		
a. Sex	6 percent	17 percent
b. Substrain	11 percent	
2. Variation due to environmental differences		
a. Litter size	4 percent	83 percent
b. Age of mother	?	
c. Miscellaneous tangible factors	6 percent	
d. Intangible factors	73 percent	

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