THE LINKAGE RELATIONS OF A NEW LETHAL GENE IN THE RAT (RATTUS NORVEGICUS)

HANS GRÜNEBERG
University College, London

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INTRODUCTION

IN A RECENT paper (GRÜNEBERG 1938), a new lethal factor has been described in the rat which kills the homozygotes at any time between birth and about the fifth week of life, the majority of the animals dying during the first two weeks. The manifestations of this gene are noticeable in many parts of the body. The gene is therefore what used to be called "pleiotropic." A detailed analysis of these "pleiotropic" effects has shown, however, that all the manifold effects can be traced back to a primary anomaly of the cartilage. Many of the cartilages, notably those of the ribs and of the trachea, undergo an enormous increase in bulk. As a consequence, a very pronounced deformity of the thorax develops. The thoracic basket becomes a completely rigid carapace, and this structure is fixed in extreme inspiration. This leads to an inflation of the lungs (emphysema), and the animals eventually die from various consequences of the emphysema. For a detailed account of this somewhat complicated case of spurious pleiotropism, the reader is referred to the original paper.

In the meantime, it has been shown by H. B. FELL and H. GRÜNEBERG (1939) that the development of the cartilage anomaly does not depend on other disturbances in the body, such as an endocrine cause, but is a peculiarity of the cartilage cells themselves.

As was already pointed out in the first communication, the new lethal gene is linked to pink-eyed yellow (*p*) and therefore carried in the albino chromosome. More data about its linkage relations have now been collected and will be reported in this paper.

THE SINGLE FACTOR RATIOS

In a total of 1782 animals from matings of two heterozygotes (tables 1–6), there were 1459 normal animals and 323 lethals. The percentage of lethals is therefore 18.13 ± 1.03 percent. This is significantly less that the expectation of 25 percent. The deficiency of lethals is almost certainly due to selective mortality during the first two or three days of life when no reliable classification is possible in the living animals. The pathological situation as described elsewhere makes it plausible that some of the lethals may die fairly soon after birth. On the other hand, the pathological mechanism of the anomaly would not point to a pre-natal elimination as a cause for the deficiency.

This is borne out by the following facts. In most cases, the number of living young was recorded within 24 hours after birth. Dividing this material in undepleted and depleted litters, the following figures are obtained.

	NORMALS	LETHALS	TOTAL	PERCENTAGE OF LETHALS
Undepleted litters	772	204	976	20.90
Depleted litters	580	105	685	15.33
Total	1352	309	1661	18.60

The percentage of lethals is therefore greater in undepleted litters, the difference being significant ($\chi^2 = 8.256$ for one degree of freedom; $P < 0.01$). It appears likely that the lethals were over-represented amongst the corpses, which were not included in the original records, and amongst the young already eaten by the mothers before the litters were discovered. The elimination of lethals, therefore, takes place largely, and probably entirely, after birth.

As is to be expected, the ratio of the marker genes linked to the lethal is secondarily disturbed, though to a lesser extent. In view of these disturbances of the ratios, the methods for the evaluation of these data have had to be somewhat modified. They are given in detail at the end of this paper in an appendix by J. B. S. HALDANE.

LINKAGE OF THE LETHAL WITH PINK-EYED YELLOW

For the estimation of the crossover percentage between these genes, several sets of data are available. In table 1 are to be found the segregations of 13 families of the constitution *pl*/++ ♀ × *pl*/++ ♂ (coupling F_2 generation). The crossover percentage, which in this case is the mean of the recombination fractions of the two sexes, amounts to 20.58 ± 2.20 percent ($I = 2063.27$).

TABLE 1

Linkage of p and l, coupling F_2 (pl/++ ♀ × pl/++ ♂).

MATING	+	*p*	*l*	*pl*	TOTAL
A	30	5	6	7	48
C	43	8	6	9	66
E	25	1	1	4	31
58	25	4	0	2	31
60	37	6	6	10	59
68	25	5	1	3	34
76	27	8	3	4	42
78	18	2	0	3	23
79	56	8	1	6	71
82	28	1	7	7	43
117	19	0	3	3	25
119	14	3	0	1	18
120	8	0	0	1	9
Total	355	51	34	60	500

Black-eyed normal offspring obtained from some of the coupling F_2 matings (A, C, and E) were mated in pairs to produce an F_3. Two such matings showed segregation both for the lethal and for *p* (table 2). Such F_3 matings may theoretically be of three different kinds. Either both parents contain the two factors in coupling (C×C), or one contains the

TABLE 2

Linkage of p and l, F_3

MATING	+	*p*	*l*	*pl*	TOTAL
22	14	2	5	3	24
27	74	8	6	5	93
Total	88	10	11	8	117

genes in coupling and the other in repulsion (C×R), or finally both animals carry the factors in repulsion (R×R). The type of mating in every individual family can only be inferred from the offspring produced. In the case of table 2, it is fairly obvious that we are dealing with C×C matings. The recombination value for these two families is $q = 21.94 \pm 4.66$ percent ($I = 461.10$). In view of the uncertainty as regards the type of these F_3 matings, a correction of the crossover value is necessary (see appendix). In this case, the correction is trivial, being of the magnitude due to the mis-scoring of a single animal.

Another set of black-eyed normal rats produced by the coupling F_2 pairs was crossed to animals of the constitution *ppl*+. This type of cross is sometimes called a single-backcross. It allows the estimation of the recombination fractions of the two sexes separately, as it can be carried out reciprocally (*p*+*l*+ ♀ × *ppl*+ ♂, table 3, and *ppl*+ ♀ × *p*+*l*+ ♂, tables 4 and 5). In either case, the black-eyed animal may carry the genes in

TABLE 3

Linkage of p and l, single backcrosses, coupling phase, female doubly heterozygous (pl/++ ♀ × pl/p+ ♂).

MATING	♀	♂	+	*p*	*l*	*pl*	TOTAL
6	C6	A2	43	37	7	14	101
8	C4	C2	26	14	0	6	46
11	E5	E3	18	9	3	8	38
13	A8	A7	42	25	4	8	79
16	C12	C11	15	7	1	5	28
17	C13	C11	23	17	3	7	50
18	C14	C11	42	24	5	8	79
51	A18	A7	22	7	2	9	40
93	A18	A2	16	10	3	9	38
54	A16	A7	6	8	2	2	18
94	A16	A2	4	1	0	1	6
Total			257	159	30	77	523

coupling or repulsion. As judged by the progeny produced, 12 out of the 13 double heterozygotes listed in tables 3–5 appear to have carried the two

genes in coupling and one in repulsion. Again, a small correction for the uncertainty of this classification will be introduced in the appendix. Add-

TABLE 4

Linkage of p and l, single backcrosses, coupling phase, male doubly heterozygous (*pl/p+* ♀ × *pl/++* ♂).

MATING	♀	♂	+	*p*	*l*	*pl*	TOTAL
19	C7	C8	24	24	3	9	60
20	A12	C17	56	32	4	15	107
42	C24	E8	26	18	2	3	49
Total			106	74	9	27	216

ing to this the four individuals of the F_3, which were classified as coupling, the ratio is 16 C:1 R. C and R double heterozygotes are obtained from a coupling F_2 in the ratio $(1-q)^2:q^2$, where q is the recombination fraction. The observed ratio of 16:1 corresponds to $q=0.20$, which is in good agreement with the estimate obtained from the coupling F_2.

In a coupling single-backcross with equal viability of all the four classes, the four phenotypes +, *p*, *l*, and *pl* are expected to occur in the ratio

$$2-q:1+q:q:1-q.$$

The product formula as given by IMMER (1930) leads to a satisfactory estimate of the crossover frequency even in those cases where this ratio is disturbed by a reduced viability of one of the genes. This is, however, not the case with IMMER'S formula for the variance of q, since those classes which give most of the information concerning q, namely the two lethal classes, are reduced in size. Hence it seems better to deal with normals and

TABLE 5

Linkage of p and l, single backcross, repulsion phase, male doubly heterozygous (*pl/p+* ♀ × *p+/+l* ♂).

MATING	♀	♂	+	*p*	*l*	*pl*	TOTAL
21*	C15	C16	8	29	3	1	41

* The ratio of black-eyed to pink-eyed is considerably distorted (11:30; $\chi^2=8.805$ for n = 1). The last litter (stillborn and therefore not classifiable for the lethal) contained five black-eyed and three pink-eyed young; if this litter is included, the ratio is 16:33 with $\chi^2=5.898$ for n = 1.

lethals separately. Two estimates for q are therefore obtained from each experiment. These values may then be combined, the weight given to each being proportional to the amount of information contained in the respective estimates.

By this treatment we obtain from the single backcross data for females

non-lethal classes $q' = 0.1466$; $I' = 195.75$

lethal classes $q'' = 0.2804$; $I'' = 530.32$

and the combined estimate is $q_{♀} = 0.2443 \pm 0.0371$ ($I = 726.07$). Similarly in the male, we obtain by combining the data of tables 4 and 5:

non-lethal classes $q' = 0.1370$; $I' = 103.39$
lethal classes $q'' = 0.2683$; $I'' = 208.85$

and the combined estimate is $q_{\sigma'} = 0.2248 \pm 0.0566$ ($I = 312.24$). The recombination fraction is, therefore, higher in females than in males. The difference is not significant in the material presented here. But, as a corresponding difference in the same chromosome of the rat has been established beyond doubt by CASTLE and WACHTER (1924), I am confident that the sex difference observed here would have become significant had the sample been sufficiently increased.

It was next desired to combine the single-backcross data with the F_2 and F_3 data. The latter are average values for crossing over in the two sexes. Now it would be inaccurate simply to pool the *data* for the two sexes; such a value would be unduly influenced by the high crossover value of the females, which would contribute more than twice as much information as the males.

Hence it seems better to combine the crossover values for the two sexes by taking the arithmetical mean of the two *estimates* and ascribing to it the standard error $\pm\sqrt{m_1^2+m_2^2}$. This leads to a joint estimate for the two sexes from the single-backcross data of $q = 0.2346 \pm 0.0310$ ($I = 1038.31$).

We now have three estimates for the crossover value of $p-l$.

F_2 data	$q = 0.2058 \pm 0.0220$	($I = 2063.27$)
F_3 data	$q = 0.2194 \pm 0.0466$	($I = 461.10$)
Single-backcross data	$q = 0.2346 \pm 0.0310$	($I = 1038.31$)
		$I = 3562.68$

As these estimates do not differ significantly, they may be combined by weighting according to the amount of information they contain respectively. The combined estimate is

$$q = 0.2160 \pm 0.0168 \quad (I = 3562.68).$$

This value, as was pointed out above, should be corrected for the fact that in the case of the F_3 and the single-backcrosses, the constitution of the double heterozygotes (whether C or R) was inferred from the offspring produced and consequently may have been faulty. The details of this correction are to be found in the appendix. It is very small and thus shows that the chance of erroneous classification of heterozygotes has been very slight in our case.

The crossover value obtained for the genes p and l has obviously to be compared with other linkage values established for this chromosome.

LINKAGE OF THE LETHAL WITH ALBINISM

The crossover value between pink-eyed yellow and albinism (c) has

been determined with considerable accuracy by CASTLE and WACHTER (1924). A recalculation of their data leads to a crossover fraction of 0.2193 ±0.00583 in females and 0.1839±0.00397 in males. The joint estimate for the two sexes obtained from these data (arithmetical mean of the estimates) is 0.2016±0.00328. The distance between pink-eyed yellow and the lethal obtained in this paper appears to be somewhat greater, the difference being 0.0144±0.0171.

Under these circumstances, the lethal factor may be either fairly close to albinism or far removed. In the former case the order of the three genes may be either $l-c \cdots p$ or $c-l \cdots p$. If far removed from albinism, the order of the genes would be $c \cdots p \cdots l$.

If c and l are close together, their distance will probably not exceed 4.86 units, that is to say, it will presumably not be greater than the difference of the crossover values $p-l$ and $p-c$ plus twice its standard error. If, on the other hand, the order of genes is $c \cdots p \cdots l$, the map distance between c and l will probably not be smaller than 38.34 units; this is the sum of the $c-p$ and $p-l$ distances less twice its standard error. Owing to undetected double crossing over, the recombination percentage will be lower. If we assume that there is no interference, the expected recombination value will be about 3.7 units less than the map distance. Actually, the assumption of an absence of interference is probably wrong, as the existence of interference has been shown in the mouse in the region $sh_1 - c-p$ of the homologous chromosome (GRÜNEBERG 1935, 1936). Hence we are on the safe side if we assume that, for the order of genes $c-p-l$, the genes for albinism and the lethal should show a recombination percentage of at least 34. It had, therefore, to be determined whether the recombination percentage between albinism and the lethal is 4.86 units or less, or whether it is 34 units or more.

For this purpose, two females homozygous for black-eyed intense fur color and heterozygous for the lethal were outcrossed to a Wistar albino male. The F_1 animals thus produced were mated in pairs and thereby tested for heterozygosity for the lethal. The matings which segregated for albinism and the lethal are given in table 6.

The data about non-lethals yield practically no information about the crossover value and can be neglected. The two lethal classes lead to an estimate of q=0.1260 (for formula see appendix, table 7). The variance of this value has not been calculated as one class is represented by a single individual only, and the distribution will hence be far from normal.

We have, therefore, to calculate the probability that only one double recessive (or none) will be found in a sample of n (=63) individuals, if the crossover value is q (q=0.33, 0.34, 0.35 etc.). This probability is

$$P = (1 - q^2)^n + nq^2(1 - q^2)^{n-1}$$

TABLE 6

Linkage of c and l, repulsion F_2 (c+/+l♀ × c+/+l♂).

MATING	+	*c*	*l*	*cl*	TOTAL
98	43	11	17	0	71
108	22	7	5	0	34
110	23	11	11	0	45
110a	43	14	6	1	64
122	21	11	8	0	40
129	29	15	3	0	47
133	18	16	7	0	41
140	19	8	3	0	30
150	5	6	2	0	13
Total	223	99	62	1	385

and yields the following values

q =	·33	·34	·35
P =	.0061	.0040	.0026

Hence even under the assumption that the expected crossover value is as low as .34, the chance of finding only one double recessive (or none) in a sample of 63 individuals is 1:250. It is, therefore, very unlikely that the order of genes is $c-p-l$.

On the other hand, the chance of finding at least one double recessive in a sample of n = 63 is

$$P = 1 - (1 - q^2)^n$$

and yields the following values

q =	.05	.04	.03	.02	.01
P =	.1459	.0960	.0551	.0249	.0063

Hence the observed ratio of 62 single recessives: 1 double recessive is compatible with the assumption that the distance between albinism and the lethal is about three to five units. We therefore conclude that the lethal gene is located close to albinism.

We have to consider next whether the order of the genes is $l-c \cdots p$ or $c-l \cdots p$. It was calculated above that the distance $p-l$ is 21.60 ± 1.68 units. It was further shown that the distance $c-l$ is unlikely to be less than three units. Now, if the lethal gene is located at least three units to the right of albinism, this would imply that the "true" distance between p and l is .1716 or less. This deviates from the observed value of .2160 $\pm$.0168 by 2.64 times its standard error or more and is therefore very unlikely ($p \leqq .0041$). On the other hand, if the lethal is to the left of albinism, the range of "allowed" values starts rather less than once the standard error removed from the observed value. We therefore conclude that the lethal gene is located to the left of albinism, and that the order of genes is $l-c \cdots p$.

We have to consider next how many units the lethal gene is to the left

of albinism. For its localization, two sets of data are available. The experiments involving *l* and *p* put the lethal 1.44 ± 1.71 units to the left of albinism. The repulsion F_2 involving *l* and *c* puts the lethal 12.6 units to the left of *c*, but this value is only a very rough one. The locus of the lethal is likely to be somewhere between these values. Now, if we assume that it is ten units to the left of *c*, this estimate would agree very well with the repulsion F_2 data (*l* and *c*), but would be exceedingly unlikely in view of the experiments involving genes *l* and *p*. Similarly, if we assume the locus of *l* to be two units to the left of *c*, this would tally very well with the *l*−*p* data, but would be unlikely from the *l*−*c* experiment. Obviously, the most likely locus of the lethal is that which is equally likely from both bodies of data.

In practice, this locus is found most easily by a process of approximation. We assume provisionally that the lethal gene is located four units to the left of *c*. Then, for the *l*−*c* experiments, $P = 1 - (1 - q^2)^n$ for $n = 63$ and $q = .04$, which works out at .0960. For the *l*−*p* data, let d_1 be the estimated distance *l*−*p* and d_2 that of *c*−*p*, and σ_{d_1} and σ_{d_2} their standard errors respectively. Then

$$\frac{0.04 - (d_1 - d_2)}{\sqrt{\sigma_{d_1}^2 + \sigma_{d_2}^2}} = \frac{0.04 - (0.2160 - 0.2016)}{\sqrt{0.0168^2 + 0.00328^2}} = 1.497.$$

This corresponds, for one tail of the distribution, to $P = 0.0672$. The provisional value of 0.04 thus agrees better with the *l*−*c* data than with the *l*−*p* data and is, therefore, too great. We try next with a distance of 3.5 units and find for the *l*−*c* and *l*−*p* data values of P corresponding to 0.0743 and 0.1141 respectively. This time, we are grossly out in the opposite direction. Interpolating, we find that for a distance of 3.8 units the probabilities are respectively 0.0870 and 0.0868. We therefore adopt the distance of 3.8 units as the final value. In view of the size of the variances involved, greater accuracy seems unnecessary.

DISCUSSION

The map of the albino chromosome of the rat now comprises five known genes, the greatest number for any mammalian chromosome so far. These five loci are

L, *l*	normal, lethal
C, c^r, *c*	fully coloured, ruby-eyed, true albino
R, *r*	" " , red-eyed yellow
P, *p*	" " , pink-eyed yellow
W, *w*	normal, waltzer

From the evidence presented by CASTLE and WACHTER (1924), KING and CASTLE (1937) and in this paper, the following map may be constructed

l	*c*	*r*	*p*	*w*
o	3.8	4.1	24.0	49 ±

It is a remarkable fact that in the rat with a haploid chromosome number of 21, five out of 13 known genes should be found in one single chromosome. The albino chromosome of other rodents is similarly well stocked with known genes. In the mouse, it contains *shaker*$_1$, *c* and *p*, in the rabbit the genes for brown fur color, yellow fat and *c*, and in Peromyscus *c* and *p*. The genes for *c* and *p* are very probably homologous in all these species. But even so, the number of genes in the albino chromosome of the rodents remains remarkable and will require a special explanation. It appears inopportune to speculate on this problem now.

Early in the experiments described in this paper, the appearance of an agouti young in the offspring of two non-agouti parents was noticed (GRÜNEBERG 1937). This was explained as very probably due to a reverse mutation. It was pointed out, however, that the young in question might have appeared as the result of segregation, if there existed in the rat a hitherto unknown gene for dominant black epistatic over and linked to agouti. In that case, more agouti young should have occurred in the experiments described here. As that has not happened, it appears virtually certain that the hypothesis of an unknown dominant black can be discounted for the case under discussion.

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SUMMARY

A new lethal mutation in the rat causes abnormalities of the cartilage followed by emphysema of the lungs and death during the first six weeks of life. The percentage of lethals in F_2 matings is reduced below expectation by selective mortality after birth, but prior to the age at which a reliable classification is possible. Coupling F_2 and F_3 generations and single backcrosses involving *l* and *p*, and a repulsion F_2 generation involving *l* and *c* lead to a localization of the lethal 3.8 units to the left of albinism.

Note Added in the Proof, July 8, 1939

Since this paper was written, more material has been accumulated. The total of table 1 is now

+	*p*	*l*	*pl*	Total
408	56	37	64	565

The crossover fraction is .2026 ± .0207 ($I = 2342.45$) as compared with .2058 ± .0220 ($I = 2063.27$). The mean crossover value for the *l*–*p* section is thereby slightly lowered from .2160 ± .0168 ($I = 3562.68$) to .2133 ± .0161 ($I = 3841.86$).

The total of table 6 is now

+	*c*	*l*	*cl*	Total
325	161	87	1	574

lowering the crossover value for the *l*–*c* section from .1260 to .1066. The chance that the order of genes is *c*–*p*–*l*, even assuming a recombination value as low as .33, is now only .00046 (formerly .0061). The most likely locus of the lethal is now 3.3 units to the left of *c*, as compared with 3.8 units before.

APPENDIX

The analysis of linkage data where the type of linkage is uncertain

J. B. S. HALDANE
University College, London

DR. GRÜNEBERG's data raise a problem which is likely to occur in a number of other cases, particularly where certain genotypes are lethal or sterile, so that the composition of double heterozygotes is uncertain. Whilst it turns out that in the particular case in question the corrections to be made are quite trivial, this will not always be so; and even in DR. GRÜNEBERG'S case it has to be shown that the uncertainty with regard to parental genotypes is unimportant.

Two types of *PpLl*×*ppLl* (or reciprocal) matings are possible, with the following expectations. Since there is a shortage of lethals, the proportions given are those of dark-eyed to pink-eyed among normals and lethals respectively.

		+	*p*	*l*	*pl*
$\frac{++}{pl}\times\frac{p+}{pl}$	(C)	$\frac{2-q}{3}:$	$\frac{1+q}{3}:$	$q:$	$1-q$
$\frac{+l}{p+}\times\frac{p+}{pl}$	(R)	$\frac{1+q}{3}:$	$\frac{2-q}{3}:$	$1-q:$	q

Here q is the crossover frequency, supposed equal in both sexes.

Similarly three types of *PpLl*×*PpLl* matings are possible, with the following expectations:

		+	*p*	*l*	*pl*
$\frac{++}{pl}\times\frac{++}{pl}$	(CC)	$\frac{3-2q+q^2}{3}:$	$\frac{2q-q^2}{3}$:	$2q-q^2:$	$1-2q+q^2$
$\frac{++}{pl}\times\frac{+l}{p+}$	(CR)	$\frac{2+q-q^2}{3}:$	$\frac{1-q+q^2}{3}:$	$1-q+q^2:$	$q-q^2$
$\frac{+l}{p+}\times\frac{+l}{p+}$	(RR)	$\frac{2+q^2}{3}:$	$\frac{1-q^2}{3}$:	$1-q^2:$	q^2

Some families can be assigned with certainty to one type. Others are doubtful. In the latter case the following procedure is adopted. We take the linkage value established on the basis of the crude data, giving each family its most probable origin, and calculate, first the probability *a priori* that a mating was of a given type, and secondly the probability that a mating of that type could have given the observed family. This yields a weighting factor.

In each case we consider a group of families consisting of:

$$\alpha+, \quad \beta p, \quad \gamma l, \quad \delta pl.$$

As the expected ratio of lethals to non-lethals is unknown, we must base our estimates on the lethals and non-lethals separately, and pool them, weighted according to the amount of information. If q_r and I_r are the estimated crossover values, and amounts of information concerning them, our final estimate is $\Sigma q_r I_r / \Sigma I_r$. Hence the values of $q_r I_r$ are needed.

TABLE 7

GROUP OF SUB-FAMILIES	q	I	qI
C non-lethals	$\frac{2\beta-\alpha}{\alpha+\beta}$	$\frac{(\alpha+\beta)^3}{9\alpha\beta}$	$\frac{(\alpha+\beta)^2(2\beta-\alpha)}{9\alpha\beta}$
C lethals	$\frac{\gamma}{\gamma+\delta}$	$\frac{(\gamma+\delta)^3}{\gamma\delta}$	$\frac{(\gamma+\delta)^2}{\delta}$
R non-lethals	$\frac{2\alpha-\beta}{\alpha+\beta}$	$\frac{(\alpha+\beta)^3}{9\alpha\beta}$	$\frac{(\alpha+\beta)^2(2\alpha-\beta)}{9\alpha\beta}$
R lethals	$\frac{\delta}{\gamma+\delta}$	$\frac{(\gamma+\delta)^3}{\gamma\delta}$	$\frac{(\gamma+\delta)^3}{\gamma}$
CC non-lethals	$1-\sqrt{\frac{\alpha-2\beta}{\alpha+\beta}}$	$\frac{4(\alpha+\beta)^2(\alpha-2\beta)}{9\alpha\beta}$	$1-\frac{4\sqrt{(\alpha+\beta)^3(\alpha-2\beta)^3}}{9\alpha\beta}$
CC lethals	$1-\sqrt{\frac{\delta}{\gamma+\delta}}$	$\frac{4(\gamma+\delta)^2}{\gamma}$	$1-\frac{4\sqrt{(\gamma+\delta)^3\delta}}{\gamma}$
CR non-lethals	$\frac{1}{2}-\frac{1}{2}\sqrt{\frac{(3(3\beta-\alpha)}{\alpha+\beta}}$	$\frac{(\alpha+\beta)^2(3\beta-\alpha)}{3\alpha\beta}$	$\frac{1}{2}I-\frac{1}{2\alpha\beta}\sqrt{\frac{(\alpha+\beta)^3(3\beta-\alpha)^3}{3}}$
CR lethals	$\frac{1}{2}-\frac{1}{2}\sqrt{\frac{\gamma-3\delta}{\gamma+\delta}}$	$\frac{(\gamma+\delta)^2(\gamma-3\delta)}{\gamma\delta}$	$\frac{1}{2}I-\frac{\sqrt{(\gamma+\delta)^3(\gamma-3\delta)^3}}{2\gamma\delta}$
RR non-lethals	$\sqrt{\frac{\alpha-2\beta}{\alpha+\beta}}$	$\frac{4(\alpha+\beta)^2(\alpha-2\beta)}{9\alpha\beta}$	$\frac{4\sqrt{(\alpha+\beta)^3(\alpha-2\beta)^3}}{9\alpha\beta}$
RR lethals	$\sqrt{\frac{\delta}{\gamma+\delta}}$	$\frac{4(\gamma+\delta)^2}{\gamma}$	$\frac{4\sqrt{(\gamma+\delta)^3\delta}}{\gamma}$

An example of the calculation is as follows. Consider the CR non-lethals. Let $x=q-q^2$. The expected values of α and β are

$$\frac{2+x}{3}(\alpha+\beta) \text{ and } \frac{1-x}{3}(\alpha+\beta). \quad \text{Hence } x=\frac{\alpha-2\beta}{\alpha+\beta},$$

$$L = \alpha \log (2 + x) + \beta \log (1 - x),$$

$$I_x = -\frac{d^2L}{dx^2} = \frac{\alpha}{(2+x)^2} + \frac{\beta}{(1-x)^2} = \frac{(\alpha+\beta)^3}{9\alpha\beta},$$

$$I_q = \left(\frac{dx}{dq}\right)^2 I_x = (1 - 4x)I_x = \frac{(\alpha+\beta)^2(3\beta-\alpha)}{3\alpha\beta}$$

$$q = \tfrac{1}{2}(1 - \sqrt{1-4x}) = \tfrac{1}{2} - \frac{3^{1/2}(3\beta-\alpha)^{1/2}}{(\alpha+\beta)^{1/2}}.$$

Hence $$qI_q = \tfrac{1}{2}I_q - \frac{(\alpha+\beta)^{3/2}(3\beta-\alpha)^{3/2}}{2\cdot 3^{1/2}:\alpha\beta}.$$

Substituting the observed values of α, β, γ, δ, we have the results of table 8, assuming that all rats have the genotype most obviously attributed to them. The weighted mean value of q is $.2213 \pm .0169$. We could, of course, estimate the value of q in the case of CC, CR, and RR matings from the value of $\alpha\delta/\beta\gamma$. This gives substantially the same result. Thus the CC figures of table 8 give $q = .215$, and $.212$ by the method of this paper. The method here given has the merit of showing that, where numbers are large, the lethals and non-lethals give much the same values of q.

TABLE 8

SUB-FAMILIES	TABLES	α, β; OR γ, δ	q	I	qI
C non-lethals	3 and 4	363,233	.2181	278.13	60.66
C lethals	3 and 4	39,104	.2727	707.21	192.63
R non-lethals	5	8,29	−.3514	24.26	−8.52
R lethals	5	3,1	.2500	21.33	5.33
CC non-lethals	1 and 2	443,61	.2023	1341.07	271.29
CC lethals	1 and 2	45,68	.2243	1135.02	254.54
			.2213	3607.02	775.93

We must now ask how far this will be changed if we allow for the fact that the genotypes of some parents may have been incorrectly given. Most of the information is derived from the F_2, where there is no uncertainty, and which gives $q = .2058$. In the case of a *PpLl* rat belonging to F_2, the *a priori* probability that it should be $++/pl$ is $(1-q)^2/(1-q)^2+q^2$, or, taking q as .2213, .9253. The probability that it should be $+l/+p$ is .07473. We have next to determine the *a posteriori* probability. In a back-cross the probabilities that $++/pl$ and $+l/p+$ rats respectively should give $\alpha+$, βp, γl, δpl offspring when crossed with $p+/pl$ are respectively:

$$\frac{(\alpha+\beta)!}{\alpha!\beta!}\left(\frac{2-q}{3}\right)^{\alpha}\left(\frac{1+q}{3}\right)^{\beta}\frac{(\gamma+\delta)!}{\gamma!\delta!}q^{\gamma}(1-q)^{\delta},$$

and

$$\frac{(\alpha+\beta)!}{\alpha!\beta!}\left(\frac{1+q}{3}\right)^{\alpha}\left(\frac{2-q}{3}\right)^{\beta}\frac{(\gamma+\delta)!}{\gamma!\delta!}(1-q)^{\gamma}q^{\delta},$$

whose quotient is

$$\left(\frac{2-q}{1+q}\right)^{\alpha-\beta}\left(\frac{1-q}{q}\right)^{\delta-\gamma}.$$

If then P is the probability that an F_2 rat which has produced such a family is $++/pl$,

$$\frac{P}{1-P}=\left(\frac{2-q}{1+q}\right)^{\alpha-\beta}\left(\frac{1-q}{q}\right)^{2+\delta-\gamma}$$
$$=1\cdot4564^{\alpha-\beta}\,3\cdot5188^{2+\delta-\gamma}.$$

$1-P$ is less than .001 except in the following families: (54+94), P = .98449; 42, P = .99887. In family 21, P = .00008.

Similarly in the case of F_3 matings the *a priori* probabilities of CC, CR, and RR as $(1-q)^4:2q^2(1-q)^2:q^4$. Hence the relative probabilities that a given family is CC, CR, and RR are:

$$\text{CC}\quad(3-2q+q^2)^{\alpha}(2q-q^2)^{\beta+\gamma}(1-q)^{2\delta+4}=\lambda P_1$$
$$\text{CR}\quad 2(2+q-q^2)^{\alpha}(1-q+q^2)^{\beta+\gamma}(q-q^2)^{\delta+2}=\lambda P_2$$
$$\text{RR}\quad(2-q^2)^{\alpha}(1-q^2)^{\beta+\gamma}q^{2\delta+4}=\lambda P_3$$

where P_1, P_2, P_3 are the actual probabilities, and λ a constant. Hence:

$$P_1/P_3=1\cdot2729^{\alpha}\times\cdot41285^{\beta+4}\times3\cdot5188^{2\delta+4}$$
$$P_2/P_3=2\times1\cdot0602^{\alpha}\times\cdot8703^{\beta+\gamma}\times3\cdot5188^{\delta+2}.$$

In family 22, $\alpha=14$, $\beta+\gamma=7$, $\delta=3$, so $P_1/P_3=17442$, $P_2/P_3=924.95$, so $P_1=.9496$, $P_2=.05035$, $P_3=.000054$. In family 27, $P_1=1.0000$, $P_2=.0000$, $P_3=.0000$.

Thus the probability of incorrect assignment nowhere exceeds 5.04 percent, and the value of q is clearly not altered appreciably. However, the corrections will be made so that the method may be applied in future cases.

A correction of $\alpha=.184$, $\beta=.158$, $\gamma=.0331$, $\delta=.0498$ must be subtracted from the C and added to the R families. A correction of $\alpha=.705$, $\beta=.101$, $\gamma=.252$, $\delta=.151$ must be subtracted from the CC families, and put as a CR family. The total correction is thus of the magnitude due to the misscoring of a single rat.

At first sight the correction cannot be made, since the value of q deduced from the CR "family" is imaginary. However, such small corrections can be made by answering the following question. "If there were already a large number of families of a given type, giving the standard value of q, what corrections to I and qI would be made as a result of the correction

TABLE 9

Formulae for small corrections.

GROUP OF SUB-FAMILIES		ΔI	ΔqI
C non-lethals		$\frac{3[(1-q)^2\alpha'+(2q-q^2)\beta']}{(2-q)^2(1+q)^2}$	$\frac{-2(1+q)(1-q+q^2)\alpha'+(2-q)(2+q+2q^2)\beta'}{(2-q)^2(1+q)^2}$
C lethals		$\frac{(1-q)(3q-1)\gamma'+q(2-3q)\delta'}{q^2(1-q)^2}$	$\frac{2(1-q)\gamma'+(1-2q)\delta'}{(1-q)^2}$
R non-lethals		$\frac{3[(2q-q^2)\alpha'+(1-q^2)\beta^2]}{(2-q)^2(1+q)^2}$	$\frac{(2-q)(2+q+2q^2)\alpha'-2(1+q)(1-q+q^2)\beta'}{(2-q)^2(1+q)^2}$
R lethals		$\frac{q(2-3q)\gamma'+(1-q)(3q-1)\delta'}{q^2(1-q)^2}$	$\frac{(1-2q)\gamma'+2(1-q)\delta'}{(1-q)^2}$
CC non-lethals	$x=(1-q)^2$	$\frac{4[2(1+x)(1+x+x^2)\alpha'-(2+x)(2-x+2x^2)\beta']}{(2+x)^2(1-x)^2}$	$\frac{\beta'}{1-x}-\frac{\alpha'}{2+x}+\frac{6q[(1-x)(2+x+x^2)\alpha'-(2+x)(2-x+x^2)\beta']}{(2+x)^2(1-x)^2}$
CC lethals	$x=(1-q)^2$	$\frac{4[(1-2x)\gamma'+2(1-x)\delta']}{(1-x)^2}$	$\frac{2\gamma'}{1-x}-\frac{2\delta'}{x}+\frac{2q[x(1-3x)\gamma'+(1-x)(1+3x)\delta']}{x(1-x)^2}$
CR non-lethals	$y=q-q^2$	$\frac{-(1-y)(5+5y+8y^2)\alpha'+(2+y)(8-7y+8y^2)\beta'}{(2+y)(1-y)^2}$	$\frac{-\alpha'}{2+y}+\frac{\beta'}{1-y}+\frac{3q[-(1-y)(3+y+2y^2)\alpha'+(2+y)(4-3y+2y^2)\beta']}{(2+y)^2(1-y)^2}$
CC lethals	$y=q-q^2$	$\frac{y(2-7y+8y^2)\gamma'-(1-y)(1-3y+8y^2)\delta'}{y^2(1-y)^2}$	$\frac{-\gamma'}{1-y}+\frac{\delta'}{y}+\frac{q[y(2-5y+6y^2)\gamma'-(1-y)(1-y+y^2)\delta']}{y^2(1-y)^2}$

to the values of α, β, γ, and δ?" This correction is independent of the original number, provided it is large.

Consider a group of C families. If there were s non-lethals, the original values of α and β being $\frac{1}{3}(2-q)s$ and $\frac{1}{3}(1+q)s$, and the corrected values $\frac{1}{3}(2-q)s+\alpha'$ and $\frac{1}{3}(1-q)s+\beta'$, then if $I+\Delta I$ is the corrected value of I, we find

$$I = \frac{s}{(1+q)(2-q)}, \qquad I + \Delta I = \frac{(s+\alpha'+\beta')^3}{[(2-q)s+3\alpha'][(1+q)s+3\beta']}.$$

Hence

$$\Delta I = \frac{3[(1-q)^2\alpha' + (2q-q^2)\beta']}{(2-q)^2(1+q)^2} \text{ approximately.}$$

Similarly

$$qI = \frac{qs}{(1+q)(2-q)}, \quad qI + \Delta qI = \frac{(s+\alpha'+\beta')^2(qs-\alpha'+2\beta')}{[(2-q)s+3\alpha'][(1+q)s+3\beta']}$$

Hence

$$\Delta qI = \frac{-2(1+q)(1-q+q^2)\alpha' + (2-q)(2+q+2q^2)\beta'}{(2-q)^2(1+q)}.$$

The formulae needed are given in table 9. Applying them to the corrections to α, β, γ, and δ we find that I must be diminished by 19.78, and qI by 1.86. Thus our final value of q is $.2220 \pm .0169$.

As the value of q is probably different in the two sexes, this value is probably too high, since more weight was given to doubly heterozygous females than males. DR. GRÜNEBERG'S value of .2160 is somewhat more plausible, but in view of the standard error of 1.7 percent no decision can be reached.

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