A METHOD OF ESTIMATING THE NUMBER OF HISTOCOMPATIBILITY LOCI IN A SIB-MATING MOUSE POPULATION¹

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Received March 29, 1963

THE immunologic and genetic bases of histocompatibility are well established. The rejection of an incompatible graft is due to an immunologic reaction of the host against the graft of the donor which contains one or more antigens lacking in the tissues of the host. The antigens involved in transplantation immunity are known to be genetically determined. These determinants, defined as histocompatibility genes, segregate according to the Mendelian principle. They act independently and determine antigens which vary in magnitude with respect to antigenicity.

Histocompatibility between two individuals depends upon their genetic and antigenic relationship. Acceptance of tissue grafts, such as skin, by one individual from another in a noninbred population has not been reported and must be an extremely rare event: this suggests that the number of histocompatibility genes is large. SNELL (1948) estimated that the number of histocompatibility genes was at least 14 in his mouse stocks. Later, BARNES and KROHN (1957) estimated that a minimum of 15 loci were segregating in the F_2 generation of a cross between two inbred strains of mice following the grafting of skin of the parental strains to animals of the F_2 generation. Recently HICKEN and KROHN (1960) estimated that the difference between A and CBA strains is due to at least ten loci.

By knowing the genetic relationship between a donor and a recipient of a graft in a given breeding system, one may estimate the number of histocompatibility genes from the frequency of successful grafts. Under these conditions, a statistical method has been derived for using a sib-mating population, in which grafts have been made between sibs, to estimate the number of histocompatibility loci in the initial pair. Both the method and illustration, using mouse data of the author, are given here.

STATISTICAL METHOD

In the case of a single locus with two different alleles, A and A_1 , in control of

¹ This investigation was supported by research grant C-3108 from the National Cancer Institute, and research grant RG-5550 from the Division of General Medical Sciences, Public Health Service. The authors are grateful to Dr. E. L. GREEN for his valuable comments on the preparation of the manuscript.

histocompatibility, there are six possible genotypic combinations of a donor and a recipient.

1. $AA - AA$
2. $AA - AA_1$
3. $AA - A_1A_1$
4. $AA_1 - AA_1$
5. $A_1A_1 - AA_1$
6. $A_1A_1 - A_1A_1$

The frequency, f_i , of each combination between sibs in a sib-mating population (see KEMPTHORNE 1955) is:

$$\begin{bmatrix} f_1 \\ f_2 \\ f_3 \\ f_4 \\ f_5 \\ f_6 \end{bmatrix} = \begin{bmatrix} 0 & -1/4 & -1/20 & -(1/2 + 2/5 \epsilon) & -(1/2 + 2/5 \epsilon') & 1/4 \\ 0 & 1/2 & 1/5 & 1/10(1 + 2 \epsilon) & 1/10(1 + 2 \epsilon') & 0 \\ 0 & 0 & -1/10 & 1/20 & 1/20 & 0 \\ 0 & 0 & -1/5 & 2/5 \epsilon & 2/5 \epsilon' & 0 \\ 0 & -1/2 & 1/5 & 1/10(1 + 2 \epsilon) & 1/10(1 + 2 \epsilon') & 0 \\ 1 & 1/4 & -1/20 & -(1/8 + 2/5 \epsilon) & -(1/8 + 2/5 \epsilon') & -1/4 \end{bmatrix} \begin{bmatrix} V_1 \\ V_2 \\ V_3 \\ V_4 \\ V_5 \\ V_6 \end{bmatrix} \quad (1)$$

where $\epsilon = .8090$, $\epsilon' = -.3090$, and

$$\begin{aligned} V_1^n &= 1 \\ V_2^n &= (1/2)^n 4pq(p^2 - q^2) \\ V_3^n &= (1/4)^n 4pq(p^2 - 3pq + q^2) \\ V_4^n &= \epsilon^n 4pq \\ V_5^n &= \epsilon'^n 4pq \\ V_6^n &= 4p \end{aligned}$$

where n = number of generations of sib-mating, and p and q are the gene frequencies for A and A_1 respectively.

Multiplying the two matrices on the right and replacing the V 's in terms of p , q , ϵ , and ϵ' , the f value for each combination of donor and recipient can be computed. To save space the computations are not given.

When one antigen is determined by gene A and the other is determined by A_1 , and each is strong enough to cause a graft to slough, animals of the same genotype, such as between two AA , two AA_1 or two A_1A_1 , will accept each other's graft. The AA animal will not accept a graft from AA_1 , but the AA_1 animal will accept a graft from AA . Between AA and A_1A_1 animals, neither will accept a graft from the other. If the failure and success for the graft to survive on the host is scored as 0 and 1 respectively, the average score (histocompatibility index) for each combination is given as

$$\begin{aligned} AA - AA &= 1.0 \\ AA - AA_1 &= 0.5 \\ AA - A_1A_1 &= 0 \\ AA_1 - AA_1 &= 1.0 \\ AA_1 - A_1A_1 &= 0.5 \\ A_1A_1 - A_1A_1 &= 1.0 \end{aligned}$$

Multiplying the probability of each combination of donor and recipient in a sib-

mating system by the corresponding histocompatibility index, the probability of successful grafts for each sib pair, s_i , can be obtained. This may be conveniently written out in a matrix equation:

$$\begin{bmatrix} s_1 \\ s_2 \\ s_3 \\ s_4 \\ s_5 \\ s_6 \end{bmatrix} = \begin{bmatrix} 1.0f_1 \\ 0.5f_2 \\ 0.0f_3 \\ 1.0f_4 \\ 0.5f_5 \\ 1.0f_6 \end{bmatrix} \quad (2)$$

Let s_{ij} = probability of a successful graft in i th genotypic combination at the j th locus ($i=1, 2, \dots, 6; j=1, 2, \dots, L$). The probability of a successful graft in generation n is

$$s_{.j} = \sum_i s_{ij} \quad (3)$$

when it is determined by one locus, and

$$s = (s_{11} + s_{21} + \dots + s_{61}) (s_{12} + s_{22} + \dots + s_{62}) \dots (s_{1L} + s_{2L} + \dots + s_{6L}) \\ = \pi_j s_{.j} \quad (4)$$

when it is determined by L loci.

The right side of equation (3) may be written out, in terms of p , q , ϵ , and ϵ' , based on equations (1) and (2):

$$s_{.j} = 1 - 2/5 (1/4)^n pq (p^2 - 3pq + q^2) + 4pq [\epsilon^n (-3/20 - 1/5\epsilon) - \epsilon'^n (-2/30 - 1/5\epsilon')] \quad (5)$$

If the gene frequencies are the same for all the loci concerned, equation (4) may be simplified:

$$s = (s_{.j})^L \quad (6)$$

$$\log s = L \log s_{.j}$$

and

$$L = \log s / \log s_{.j} \quad (7)$$

We may keep in mind that in a sib-mating system a line begins from one pair of ancestors at the zero generation, and the hypothesis of two alleles at each histocompatibility locus. Of the two ancestors at Generation 0, the histocompatibility loci which have segregated at later generations must be either one of the following types: AA vs A_1A_1 , AA_1 vs AA_1 , AA vs AA_1 , or A_1A_1 vs AA_1 . The first and second types give a gene frequency of .50 for both A and A_1 . Since it has been generally considered that there is no dominance among histocompatibility genes, the third and fourth types can be considered as one class with gene frequencies of .75 for one allele and .25 for the other. When the p and q values are replaced by .50 in equation (5), $s_{.j}$ can be computed in terms of ϵ , ϵ' , and n .

$$s_{.j} = 1 + 1/10 (1/4)^{n+1} - .312 \epsilon^n - .088 \epsilon'^n \quad (8)$$

The second and fourth terms on the right side of (8) diminish rapidly with the increase of n , and can be dropped out when n is greater than 2. Therefore, equation (8) can be written, in terms of ϵ and n , for one locus, as

$$s_{.j} \approx 1 - .312 \epsilon^n \quad (9)$$

$$\text{and for } L \text{ loci as } s \approx (1 - .312 \epsilon^n)^L \quad (10)$$

Based on the observed percentage of successful grafts, s_o , the number of loci, L , can be estimated:

$$\hat{L} \approx \log s_o / \log (1 - .312 \epsilon^n). \quad (11)$$

Similarly, putting .75 and .25 for the p and q values respectively in equation (4), the following equations can be given. For one locus:

$$s_{.j} = 1 - 6/5 (1/4)^{n+1} - .234 \epsilon^n - .066 \epsilon'^n \quad (12)$$

$$s_{.j} \approx 1 - .234 \epsilon^n \quad (13)$$

and for L loci

$$s \approx (1 - .234 \epsilon^n)^L \quad (14)$$

and

$$\hat{L}^* \approx \log s_o / \log (1 - .234 \epsilon^n). \quad (15)$$

By using equations (11) and (15) and by knowing the percentage of successful grafts, s_o , in an inbred generation, n , one can estimate the number of loci segregating in the two individuals from which a sib-mating population is derived. The use of these equations is discussed further in the following sections.

ILLUSTRATION

LG and C57BR/cdJ mice were used for the experiment. The LG mice were developed by selection for large body size for an unknown number of generations by H. D. GOODALE. In 1948 some of these mice were obtained by DR. M. N. RUNNER of the Jackson Memorial Laboratory. He sib-bred them for four or five generations, and then, in an attempt to increase vigor, inter-family matings were made. The first author obtained some of the crossbred progeny from RUNNER and resumed sib-mating. For the purpose of this study these mating pairs were considered to be in the zero generation of inbreeding, but inbreeding of some slight extent must have occurred in the past. A more detailed account of the history of development of the LG mice has been reported elsewhere (CHAI 1956). The C57BR/cd mice were obtained from the Production Department of the Jackson Memorial Laboratory.

Skin grafts were made in the LG mice, beginning from the 13th generation and continuing through the 19th generation of inbreeding. All the mice used traced back to a single mating at generation zero. The donors and recipients in each generation were sibs, and whenever possible reciprocal grafts were made between members of the same sex. In the case of different sexes, the females were always used as donors. The grafts were taken from the ear skin by cutting off about two-thirds of the external ear, and the cuts were then trimmed to a round shape and the skin separated from the cartilage. The sites of grafting were on either or both sides of the chest posterior to the elbow of the front legs. The general operations were performed according to the ordinary procedures used in skin grafting. First observations were made on the eighth day after grafting and continued through 90 days. Within three weeks after grafting the grafts were examined once every day. Later, depending on the condition of the grafts, they were examined once from every two days to a week. The day on which the grafts showed definite signs of breakdown was recorded as the date of slough, and was

used to calculate the survival time of the grafts. All the mice used in this experiment were between three months and one year old.

The total number of grafts, the percentage of acceptance, and the average survival periods, and standard deviations for the sloughed grafts are given in Table 1, for seven generations of the LG strain. There was a marked increase in the percentage of acceptance of skin grafts from the 14th to the 17th generation. The average number of days of survival for the sloughed grafts and the standard deviations vary greatly among the generations, although there may be a tendency for both to decrease in the late generations.

There were 21 grafts made in the C57BR/cdJ mice and among them 19 (90.5 percent) were accepted. The two mice which sloughed the grafts were grafted again, using the same donors, and they accepted the second grafts. The 9.5 percent of slough of the first grafts is considered as technical error, although it is realized that percentage of rejection of autografts in the LG mice may be a better control of technical error and should be available. We will use 10 percent as the rate of technical error.

The values obtained for $\hat{L}$ are given in Table 2. The number of histocompati-

TABLE 1

Percentages of successful grafts and mean survival of the rejected grafts in the LG mice from the 13th to 19th generation of sib-mating

Generation	Number of mice grafted	Percent accepted	Rejected grafts	
			Mean survival days	Standard deviation
13	17	47.1	22.8	5.76
14	86	46.5	31.3	17.92
15	88	64.0	35.4	32.60
16	59	72.9	21.3	11.93
17	23	87.0	32.0	34.64
18	22	72.7	16.2	6.14
19	17	88.2	17.5	.71

TABLE 2

Estimates of number of segregating loci at 0 generation of sib-mating in the LG mice based on the percentages of successful grafts in generations from 13 to 19 and p values of .50 and .75

Generation	p = .50		p = .75	
13	37.28	27.75*	50.15	36.98*
14	47.50	35.42	63.46	47.32
15	34.12	23.02	45.82	30.91
16	28.59	16.97	39.90	23.68
17	15.39	3.37	21.68	4.74
18	45.40	27.04	61.27	36.50
19	20.89	3.02	29.80	4.31
Weighted mean†		14.6		20.7
Standard deviation		11.3		15.2

* Correction for 10 percent technical error.

† See text for weighting.

bility loci segregating at Generation zero in this breeding system were estimated in two ways, both based on the actually observed percentages of successful grafts from the 13th to 19th generation. One is based on gene frequencies of 0.50 and the other on 0.75. In a similar fashion, the $\hat{L}$ values are computed after a correction for the 10 percent technical error. A mean and its standard deviation are computed for each of the latter two sets of estimates and given in the last two rows of the table. Since the estimates are based on logarithms of percentages which have different variances and the numbers of grafts are different in the generations, the estimates are weighted in computing the means and standard deviations: $\hat{L} = \Sigma NW \hat{L} / \Sigma NW$, and variance of $\hat{L} = [\Sigma NW \hat{L}^2 - (\Sigma NW \hat{L})^2 / \Sigma NW] / \Sigma NW - 1$, where N is the number of grafts in each corresponding generation and the weights, $W = s_0 / (1 - s_0)$. (The variance of a function, $\sigma_{f(y)}^2 = [f'(\gamma)]^2 \sigma_y^2$ (KEMPTHORNE 1952). (This equation is an approximation.) Therefore, variance of $\log s_0 = (\frac{d}{ds_0} \log s_0)^2 \text{var. } s_0 = 1/s_0^2 [s_0(1 - s_0)] = (1 - s_0)/s_0$. Consequently, $W = s_0/(1 - s_0)$. As an approximation, each s_0 , in this case, is the intercept of each inbred generation on the provisional regression line which was established from the plots of logarithms of the percentages of survived grafts against the number of inbred generations on an ordinary graph paper.)

On the two-allele hypothesis, it is unlikely that the p values were either all .75 or all .50. The estimate of 20.7 ± 15.2 based on $p = .75$ gives possibly the upper limit and that of 14.6 ± 11.3 based on $p = .50$ the lower limit. The actual value should be somewhere between these two limits under the specified conditions. We take the mean of these two means, 18, as the best estimate of the number of segregating loci in the original pair.

DISCUSSION

The method of estimating the number of loci with genes segregating in a sib-mating population here presented is based on the hypothesis of two alleles in each locus, no linkage, and no selective advantage in favor of heterozygotes. If any of these conditions is not true, the rate of fixation will be smaller, $\varepsilon > .8090$, and the value of the denominators in the equations (11) and (15) increases algebraically. Therefore for the same percentages of surviving grafts and the same number of generations of inbreeding, smaller L values would be obtained than those obtained when these conditions do not exist. On the other hand, if selection in favor of either of the homozygotes exists in the breeding system, $\varepsilon < 0.8090$, then a larger L value would be obtained. Furthermore, if there are genes of which a single dose difference is not strong enough to cause the rejection of a graft, the histocompatibility indices for the $AA-AA_1$ and $A_1A_1-AA_1$ pairs would be greater than 0.5 but smaller than 1.0, and for the $AA-A_1A_1$ pair, greater than 0 but smaller than 0.5. Under this condition, smaller values for the constants in equations (11) and (15) would result. Therefore a larger L value would be obtained, and the present equation would give an underestimate for the number of loci determining the histocompatibility.

Selection in favor of heterozygotes in blood group genes was reported in chickens by BRILES and ALLEN (1961) but has not been reported in mice. A selection coefficient as low as .76 (HAYMAN and MATHER 1953) for the homozygotes would be sufficient to hold a sib-mating population in equilibrium with respect to the level of heterozygosity and would prevent complete fixation.

Theoretical values of percentages of successful grafts between sibs have been computed based on equations (1) and (14) for 1, 10, 30, and 50 loci in a sib-mating population propagated from a single pair. These values are plotted and given in Figure 1. Under the assumptions given, these values give the upper ($p = .75$) and lower boundaries ($p = .50$) for the different number of loci assigned with respect to the generations of inbreeding. According to equation (8), the percentage of successful grafts is about 62.5 at Gen-

FIGURE 1.—Diagrams showing the theoretical values of percentages of successful grafts in a sib-mating population with histocompatibility loci of 1, 5, 10, 30, and 50 in segregation under the assumed conditions. The upper graph is made based on $p = .50$ for all the loci and equation (10), and the lower graph on $p = .75$ and equation (14).

eration 0 of inbreeding for difference at one histocompatibility locus. This value is the same as that obtained by NEWTH (1962) for random pairs. The same value can be obtained by summing the products of each frequency of combination and compatibility index, in a randombred population, for $p = .50$, and serves as a check of the present method. The computation for this is given in Table 3. The equations (11) and (15) can be used to estimate loci segregating in any generation from 0 up to the last generation by subtracting from n the number of the generation, x , at which the number of segregating loci is to be estimated, that is, in the case of $p = .50$, $\hat{L} = \frac{\log s_0}{\log [1 - .312 \epsilon^{n-x}]}$, and in the case of $p = .75$, $\hat{L}^* = \frac{\log s_0}{\log [1 - .234 \epsilon^{n-x}]}$

Based upon the genetic relationships and the compatibility indices for each genotypic combination of donor and recipient, equations can be derived for other types of inbreeding systems and for alleles when there are more than two at a locus. Other types of inbreeding in experimental animals are employed much less frequently than sib-mating, consequently these equations would be of very limited use.

The estimates of SNELL (1948) and of BARNES and KROHN (1951) give the number of histocompatibility loci by which two inbred strains differed. The value obtained in the present illustration is an estimate of the number of loci by which the two random individuals from a mouse stock selected for large body size differed. There is no reason to expect these values to be comparable in magnitude. The present value appears to be slightly greater than their estimates. The actual value should be still greater, because loci with fixed identical genes are not included. The increase in percentage of successful skin grafts demonstrates an increase in fixation with the advance of inbreeding in a sib-mating mouse population. The larger standard deviations for graft survival time in some generations than in others possibly indicate segregations of both strong and weak histocompatibility genes.

TABLE 3

The expected percentage of surviving grafts in a randombred population, $p = .50$, obtained by summing the products of each frequency (of donor and recipient) and the corresponding histocompatibility index

Frequency of recipient	Frequency of donor			Total
	1/4 AA	1/2 AA ₁	1/4 A ₁ A ₁	
1/4 AA	$(1/4)^2 \times 1 = 1/16$	$1/4 \times 1/2 \times 0 = 0$	$1/4 \times 1/4 \times 0 = 0$	1/16
1/2 AA ₁	$1/2 \times 1/4 \times 1 = 1/8$	$1/2 \times 1/2 \times 1 = 1/4$	$1/2 \times 1/4 \times 1 = 1/8$	4/8
1/4 A ₁ A ₁	$1/4 \times 1/4 \times 0 = 0$	$1/4 \times 1/2 \times 0 = 0$	$(1/4)^2 \times 1 = 1/16$	1/16
Total	3/16	1/4	3/16	10/16

SUMMARY

Equations were derived for estimating the number of segregating histocompatibility loci in the initial pair from which a sib-mating population originated, using the theory of inbreeding by sib-mating and the percentage of successful grafts between sibs. It was assumed that there were two alleles at each locus, that there was no linkage and no selective advantage in favor of heterozygotes, and that a single gene difference caused the rejection of a graft. The method was illustrated using data of skin grafts between sibs in a sib-mating mouse population.

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