LINKAGE STUDIES WITH NINE BLOOD-GROUP LOCI IN PIGS¹

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FOURTEEN blood-group systems have been reported in pigs (NIELSEN 1961; ANDRESEN 1962, 1963; ANDRESEN and BAKER 1964; HÁLA and HOJNÝ 1964; HOJNÝ and HÁLA 1965; RASMUSEN 1964). Two of these systems, C and J, are closely linked (ANDRESEN and BAKER 1964; RASMUSEN 1965; ANDRESEN 1966); and IMLAH (1965) has detected a close linkage between the locus for hemoglobin-binding proteins (KRISTJANSSON 1961) and the K blood-group system. Close linkage has been excluded between any two of the first nine blood-group systems discovered (ANDRESEN 1963). The possibility of detecting loose autosomal linkage or sex-linkage relative to these nine systems is considered in this report.

MATERIALS AND METHODS

The linkage data summarized in Table 1 are based on results of blood-typing pigs of three breeds. The Landrace pigs comprised 120 progeny groups, with four full-sibs in each group. Results of blood-typing these pigs were reported previously (ANDRESEN 1963). There are 81 litters of Duroc pigs and 68 litters of Hampshire pigs, with 3 to 16 pigs in each litter. Details of blood-typing procedures are given elsewhere (ANDRESEN 1962, 1963).

Morton's lod score method: The term "lod" is derived by elision of *logarithm of the odds*; *u* score method as described by FINNEY were used for the linkage analyses (FISHER 1935; FINNEY 1940). Only double backcross (= testcross, $Gg Tt \times gg tt$) matings were studied.

Morton's lod score method: The term "lod" is derived by elision of *logarithm of the odds*; that is, *lod* is the log of the probability ratio, $1/P$. For example, *lod* score 3 corresponds to $\log 1/P$ where $1/P = 10^3$; i.e., $P = 0.001$ (cf. Figure 1). Each family was scored by using the *lod* score functions z_1 or $z_1 + e_1$. Correction factors (e_1) were omitted for litter sizes greater than seven. *Lod* scores corresponding to recombination fractions $\theta_1 = 0.01, 0.02, \dots, 0.49$ were calculated for each double backcross mating. The scores for each value of θ_1 were subsequently summed over all families (matings) within each of the 45 possible comparisons of nine blood-group loci and sex.

For human linkage data MORTON states that linkage has been detected if the cumulative score $Z (= \sum z)$ is greater than 3 for any chosen value (θ_1) of θ . If $Z < -2$ one may conclude that the recombination fraction is significantly greater than θ_1 . Finally, if $-2 < Z < 3$, judgment about linkage should be suspended until further data lead to a decision. These significance levels are chosen because of the low prior probability of linkage. The same reasoning applies to pigs, since they have 18 pairs of autosomes (McCONNELL, FECHHEIMER, and GILMORE 1963). Hence, the chance that two random loci are linked is only about 5%.

Fisher's u score method: The families considered in this study, i.e., double backcross matings, correspond to the so-called "certain" families of Mating Type 13, 16 and 20 given in FINNEY's Table 1. The score for each family (mating) is given by $\lambda - \bar{\lambda}$, and the statistical information is κ .

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TABLE 1

Lod scores (Z)† and u scores for testing linkage relative to nine blood-group loci and sex in pigs.
 The data consist of all double backcross matings observed among a total of 269 matings

Comparisons	Number of		Z for $\theta_{1/2}$				Value of		u score $u = \Sigma(\lambda - \bar{\lambda})$	Infor- mation $\Sigma(\kappa)$
	Matings	Sibs	.10	.20	.30	.40	θ_1 for $\hat{Z} =$ approx. —2	$Z\%$		
A-E	16	80	—7.29	—2.04	—0.26	0.09	.20	0.09	10	182
F	6	25	—1.69	—0.42	—0.03	0.02	.08	0.03	2	43
G	10	65	—5.14	—1.28	—0.08	0.09	.17	0.10	8	204
H	19	88	—12.02	—4.81	—1.73	—0.38	.28	0	—20	176
I	15	59	—5.67	—1.85	—0.46	—0.05	.19	0	—1	89
J	4	30	—3.96	—1.50	—0.49	—0.10	.17	0	—5	99
K	12	60	—5.89	—2.01	—0.57	—0.09	.20	0	—4	145
L	7	46	—6.19	—2.40	—0.82	—0.17	.21	0	—9	149
Sex	37	187	—19.24	—6.39	—1.62	—0.17	.28	0	—3	445
E-F	27	197	—22.69	—7.41	—1.62	0.02	.28	0.07	17	789
G	57	423	—64.74	—25.54	—8.70	—1.67	.39	0	—79	1625
H	56	321	—41.43	—15.27	—4.73	—0.78	.35	0	—35	976
I	31	167	—18.84	—6.48	—1.72	—0.17	.29	0	—1	449
J	23	181	—18.22	—5.40	—0.89	0.12	.26	0.12	17	699
K	67	380	—38.64	—12.47	—2.86	—0.09	.31	0.05	19	1125
L	39	280	—22.50	—5.01	0.50	0.91	.23	1.13	88**	1016
Sex	104	650	—78.10	—28.00	—8.25	—1.24	.38	0	—47	2155
F-G	17	127	—14.86	—5.20	—1.46	—0.20	.27	0	—7	459
H	14	112	—14.10	—5.13	—1.56	—0.26	.28	0	—12	463
I	10	50	—3.42	—0.66	0.14	0.15	.13	0.20	12	127
J	10	87	—12.15	—4.72	—1.60	—0.33	.28	0	—17	361
K	13	90	—12.62	—4.94	—1.69	—0.33	.28	0	—17	341
L	15	109	—18.37	—7.50	—2.69	—0.57	.32	0	—30	388
Sex	32	286	—31.80	—9.99	—1.84	0.27	.29	0.29	49	1293
G-H	29	194	—21.46	—7.06	—1.68	—0.11	.29	0.004	3	675
I	8	49	—4.80	—1.53	—0.35	—0.02	.17	0.002	1	151
J	18	155	—21.24	—7.95	—2.44	—0.35	.31	0	—9	641
K	33	239	—21.86	—6.07	—0.65	0.38	.26	0.39	44	882
L	22	181	—21.97	—7.62	—2.02	—0.19	.30	0	—1	707
Sex	42	309	—35.22	—11.86	—2.90	—0.13	.31	0.04	17	1177
H-I	13	60	—6.44	—2.29	—0.70	—0.12	.21	0	—6	138
J	11	97	—5.19	—0.16	1.02	0.58	.14	1.03	46*	414
K	30	136	—12.52	—3.95	—0.93	—0.08	.25	0	—1	271
L	9	65	—4.88	—1.06	0.05	0.13	.16	0.17	11	235
Sex	65	352	—29.01	—7.54	—0.56	0.53	.26	0.58	54*	1006
I-J	6	54	—7.00	—2.58	—0.81	—0.14	.22	0	—7	231
K	17	97	—9.98	—3.03	—0.50	0.10	.22	0.10	15	307
L	12	86	—10.56	—3.78	—1.10	—0.16	.25	0	—6	329
Sex	25	133	—12.60	—3.87	—0.78	0.00	.24	0.03	6	356
J-K	10	90	—8.31	—2.20	—0.12	0.21	.20	0.23	22	420
L	7	66	—7.11	—2.27	0.51	—0.03	.21	0.001	1	291
Sex	27	245	—30.18	—10.82	—3.18	—0.49	.33	0	—20	1110
K-L	20	136	—17.98	—6.59	—1.96	—0.29	.29	0	—10	512
Sex	88	579	—60.50	—18.94	—3.80	0.15	.32	0.22	47	2023
L-Sex	30	242	—32.32	—11.80	—3.46	—0.45	.33	0	—10	1016

Correction factors ($\bar{\lambda}$) were omitted for litter sizes greater than seven. The score, and also the information, are summed over all families for each linkage relationship. If there is linkage, the sum of scores is positive and becomes greater as more data are collected. The expected value of the sum of scores is zero in the absence of linkage. The standard error of the score equals the square root of the information. Therefore, FINNEY (1940) states that linkage has been demonstrated at the 5% level of probability if $\Sigma(\lambda - \bar{\lambda})$ is greater than $1.64 (\Sigma\kappa)^{1/2}$, and at the 1% level if $\Sigma(\lambda - \bar{\lambda})$ is greater than $2.33 (\Sigma\kappa)^{1/2}$.

RESULTS AND DISCUSSION

A summary of *lod* scores for selected recombination values, θ_1 , is presented in Table 1. The maximum value, $\hat{Z}$, of the accumulated *lod* scores is shown for each of the 45 comparisons. There is no significant evidence of linkage because none of the $\hat{Z}$ values exceed the significance level of $Z = 3$ ($P = .001$) recommended by Morton. Since $Z = \log_{10}$ of odds and $P = 1/\text{odds}$, the values of $\hat{Z} = 1.13$ and $\hat{Z} = 1.03$ for the E-L and H-J comparisons correspond to probabilities of $.05 < P < .10$. The value of $\hat{Z} = 0.58$ for the H-Sex comparison corresponds to $P = .26$.

The *lod* score curves corresponding to the three highest $\hat{Z}$ values just considered are depicted in Figure 1. For comparison, three additional curves are shown to illustrate examples of close linkage (C-J) and very loose or no linkage (C-Sex, J-Sex) (ANDRESEN 1966). The curve for each of the 42 remaining comparisons can easily be constructed because four concomitant values of θ_1 and Z are given in Table 1 for each comparison (Note that $Z = 0$ for $\theta_1 = .50$).

Although homogeneity tests can be applied with advantage even in the absence of any hint of linkage, such tests are particularly appropriate in this instance because the three highest $\hat{Z}$ values correspond to the three significant u scores described in a following section. According to MORTON (1962), a convenient homogeneity criterion is the logarithm of the likelihood ratio,

$$\mathfrak{L} = 2(\log_e 10) \left(\sum_{i=1}^n \hat{z}_i - \hat{Z} \right) = 4.605(\Sigma \hat{z}_i - \hat{Z}),$$

where $\hat{z}_i$ denotes the maximum of z_i and the index letter refers to the i th pedigree. As described before, $\hat{Z}$ is the maximum of $Z(= \Sigma z_i)$ in the same interval, $0 \leq \theta \leq 1/2$, and n is the number of pedigrees. Since $\mathfrak{L}$ has the chi-square distribution with $n - 1$ degrees of freedom if the data are homogeneous, the three χ^2 values are:

$$\begin{aligned} \chi^2_{\text{E-L}} &= 4.605(10.14 - 1.13) = 41.5 \text{ for 38 df; } .30 < P < .40 \\ \chi^2_{\text{H-J}} &= 4.605(3.18 - 1.03) = 9.9 \text{ for 10 df; } .40 < P < .50 \\ \chi^2_{\text{H-Sex}} &= 4.605(9.15 - 0.58) = 39.5 \text{ for 64 df; } .99 < P < .995 \end{aligned}$$

Table 1 footnotes:

• $P < 0.05$; ** $P < 0.01$.

†, ‡ Z = cumulative *lod* score, Σz_i ; $z_i = z_1$ or $z_1 + e_1$ (MORTON 1955).

§ $\hat{Z}$ = maximum of Z .

FIGURE 1.—Diagrams of the accumulated *lod* scores (Z) corresponding to the linkage-relationships H-J, E-L, and H-Sex. Three previously reported diagrams, illustrating close autosomal linkage, are shown for comparison.

The three χ^2 values indicate that the evidence for joint segregation is based on homogeneous data. However, as mentioned in MATERIALS AND METHODS, judgment about linkage should be suspended until further data lead to a decision, because the maximal value, $\hat{Z}$, of the accumulated *lod* scores is less than 3 for each of the three comparisons.

The value of θ_1 corresponding to the accumulated *lod* score Z equal to approximately -2 is indicated in Table 1 for each of the 45 comparisons. Since $Z = -2$ corresponds to a probability of 1%, the results show that linkage closer than $\theta = .15 - .30$ has been excluded in most instances with an assurance corresponding to the 1% significance level.

The 45 u scores and the corresponding information are shown in the last two columns of Table 1. Negative values of u are not suggestive of linkage. Among the positive scores, three are significantly greater than zero. The u score for the E-L comparison is significant at the 1% level, and the u scores for the H-J and H-Sex comparisons are significant at the 5% level. The score for the H-Sex comparison refers to the possibility of detecting partial sex-linkage, because the existence of heterozygous males precludes complete sex-linkage of the H locus (ANDRESEN 1964).

In a previous study based on data from pigs of the Landrace breed, the chi-square value for linkage between the *H* and *J* loci corresponds to a probability of $.10 < P < .20$, and the *u* score for linkage between the *E* and *L* loci corresponds to a probability of $.50 < P < .10$ (ANDRESEN 1963, Tables 45 and XII). In that study, however, the *u* scores were merely applied to a few of the Landrace data mentioned in MATERIALS AND METHODS. Hence, all double backcross matings encountered in those data are analyzed in this report.

The evidence for linkage by *lod* scores and by *u* scores corresponds well, based on the data from the three breeds. The three highest *u* scores correspond to the three highest *lod* scores. The *lod* scores were not significant, in contrast to the *u* scores. However, *lod* scores and *u* scores are based on different hypotheses (H_1) alternative to the same null hypothesis (H_0). Linkage tests using *u* scores consist in testing the null hypothesis H_0 that $\theta = \frac{1}{2}$ against the alternative hypothesis H_1 that $0 < \theta < \frac{1}{2}$. The *lod* score method is used for testing the same null hypothesis ($\theta = \frac{1}{2}$) against the alternative hypothesis H_1 that $\theta = \theta_1$.

According to SMITH (1953) false claims of linkage by using *u* scores would be avoided by using "a more stringent significance level, say .001 instead of .05." The *u* scores are appropriate for the purpose stated for this investigation because they are statistically efficient in the limit for loose linkage (FISHER 1935). The efficiency tends to zero, however, for very close linkage. In the continuing search for linkage in pigs, it will be desirable to apply tests that are efficient for detecting close ($\theta < .10$) and moderate linkage ($\theta = .10 - .20$) and at the same time are efficient, or nearly efficient, for loose linkage ($\theta > .20$). Sequential tests have such properties, and they can be used for both detecting and estimating linkage, whereas the alternative scoring systems, including the *u* scores, are mainly useful in the detection of linkage. The latter is also true for chi-square tests, but, contrary to the *u* score and *lod* score methods, the χ^2 method usually is not effective in detecting linkage, if only small families are available (MATHER 1951). MORTON (1955, 1962) has stressed the additional advantages of the sequential linkage test. One such advantage is evident from this report; that is, without the need for calculating the variance, *lod* scores provide direct information about the extent to which linkage has been excluded.

SUMMARY

MORTON's *lod* score method and FISHER's *u* score method were applied to assess linkage relationships relative to nine blood-group loci in pigs. Significant evidence of partial sex-linkage of the *H* locus and also of linkage between the *E* and *L* loci and between the *H* and *J* loci was obtained by the *u* score method. However, this evidence for linkage was not confirmed by the *lod* score method; nor was there any significant evidence of linkage among the remaining 42 comparisons of blood-group loci and sex using the *lod* score method. Linkage closer than $\theta = .15 - .30$ was excluded in most instances with an assurance corresponding to the 1% significance level.

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