THE DETERMINATION OF LINKAGE INTENSITIES FROM F_2 AND F_3 GENETIC DATA INVOLVING CHROMOSOMAL INTERCHANGES IN BARLEY[1]

W. D. HANSON[2] AND H. H. KRAMER

Purdue University Agricultural Experiment Station, Lafayette, Indiana

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THE calculation of linkage values between a qualitative factor pair and the point of interchange between two non-homologous chromosomes is relatively easy when backcross data are available. In crosses to a standard normal, partial sterility behaves as a dominant character with segregation for both the factor pair and sterility occurring in a 1:1 ratio. In a naturally self-pollinated species such as barley, the use of backcross procedures to obtain linkage intensities is not feasible which necessitates the utilization of segregating F_2 and F_3 data. In F_2 data sterility gives a ratio of 1 standard normal:2 semisterile:1 homozygous interchange normal. Since the normal classes are indistinguishable, a phenotypic ratio of 1:1 is expected for the sterility classification, while a completely dominant qualitative factor pair will give the expected 3:1 ratio. JOACHIM (1947) has shown that in such cases special formulas are necessary and has presented tables to facilitate the calculation of the linkage intensities by the product method and of the corresponding standard errors.

The separation of certain F_2 phenotypes into genotypes in F_3 rows will give additional information regarding linkage. In such cases it would be desirable to obtain a method for the determination of linkage from F_3 data and to combine this information with that obtained from the F_2. The purpose of this paper is to develop such a method for obtaining linkage values from different sources of segregating data involving interchanges and to devise a means of combining these sources of information. Data obtained from a study involving the segregation of partial sterility due to a chromosome interchange and certain qualitative factor pairs in barley will be used to illustrate this method. Although a partial sterility of approximately 25 percent is exhibited in barley, the term of semisterility will be used to designate individuals heterozygous for an interchange.

DERIVATION OF FORMULAS

Genetic Expectation

An F_1 individual heterozygous for an interchange (TN) and for a gene pair (Aa) will produce four types of viable gametes AT, aT, AN, and aN, where the symbols T and N are used to designate the interchange and the normal pairs of chromosomes respectively. Assuming that the recombination value p

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[2] Present address: Department of Agronomy, University of Florida, Gainesville, Florida.

is the same in the two sexes, the gametic frequencies where *a* is carried by the parent homozygous for the interchange will be:

F_1 gametes	*AT*	*AN*	*aT*	*aN*
Gametic frequency	p/2	(1−p)/2	(1−p)/2	p/2

In the F_2 a *TN* zygote will give rise to a semisterile individual (SS) while plants from *TT* or *NN* zygotes will be normal (N) and indistinguishable. The F_2 phenotypes and the corresponding expected frequencies which may be tabulated from a Punnett square are:

F_2 Phenotype	*A*SS	*A*N	*a*SS	*a*N
Phenotypic frequency	$\frac{2-2p(1-p)}{4}$	$\frac{1+2p(1-p)}{4}$	$\frac{2p(1-p)}{4}$	$\frac{1-2p(1-p)}{4}$

Only the dominant *A* phenotypes will furnish further information regarding linkage in the F_3. In the separation of the F_2 phenotypic classes into genotypes the following frequencies are expected:

	AA	*Aa*	TOTAL
Semisteriles	$\frac{p(1-p)}{2}$	$\frac{1-2p(1-p)}{2}$	$\frac{1-p(1-p)}{2}$
Normal	$\frac{1-2p(1-p)}{4}$	p(1−p)	$\frac{1+2p(1-p)}{4}$
Total	1/4	1/2	3/4

The expression p(1-p) appears in all of the expected F_2 and F_3 frequencies. Thus, the expected frequencies remain the same whether the dominant gene enters the cross from the parent carrying the normal chromosomes or from the interchange parent, and coupling and repulsion phases can not be detected by phenotypic classification. Any formula derived from these expected frequencies must be applicable to either genetic cross.

Since all expected frequencies can be expressed in terms of p(1-p), recombination and non-recombination gametes must lose their identity as the result of F_2 and F_3 phenotypic classification of interchange data. This would mean that, essentially, the frequency of the recombination gamete can be determined only indirectly in formulas employing these expected frequencies. This limitation becomes apparent after a consideration of the zygotic frequencies tabulated with respect to the genotypes and the gametic origin as presented in table 1. All *AA* or *aa* normal zygotes and the *Aa* semisterile zygotes result from the union either of recombination or non-recombination gametes. However, the ratio of the recombination to the non-recombination union of gametes is such that each of the three genotypic classes reduces to terms of the product of the respective gametic frequencies. The *Aa* normal and the *AA* or *aa* semisterile zygotes result only from the union of a recombination gamete × non-recombination gamete. Thus, the frequency of the recombination gametes can not be studied directly; rather the parameter which can be measured directly is the product of the two respective frequencies, p(1-p).

Further, as a result of this limitation, no information could exist concerning the recombination gametes that would be entirely free from confounding by

the non-recombination gametes. At 50 percent recombination (table 1), recombination gametes are completely confounded with non-recombination gametes and little if any information regarding their identity is available. This has been illustrated by JOACHIM (1947) in her table 3 which shows that no information is available at p = .5, as computed by FISHER's general formula for this quantity. Recombination values other than .5 are reflected indirectly by deviations from a 3:1 ratio of *A* to *a* phenotypes in both the F_2 normal and semisterile classes and by the deviation of the *Aa* and *AA* genotypes from a 2:1 ratio. However, since the recombination and non-recombination values are not genetically separable, the deviation must be considered as a function of both.

TABLE 1

F_2 genotypic frequencies in normal and semisterile classes in relation to gametic origin.

PERCENT RECOMBINATION	GAMETES* COMBINED	NORMAL			SEMISTERILE		
		AA	*Aa*	*aa*	*AA*	*Aa*	*aa*
p	N×N	$(1-p)^2/4$	—	$(1-p)^2/4$	—	$(1-p)^2/2$	—
	N×R	—	$p(1-p)$	—	$p(1-p)/2$	—	$p(1-p)/2$
	R×R	$p^2/4$	—	$p^2/4$	—	$p^2/2$	—
50	N×N	.06	—	.06	—	.12	—
	N×R	—	.25	—	.12	—	.12
	R×R	.06	—	.06	—	.12	—
0	N×N	.25	—	.25	—	.50	—

*N = Non-recombination and R = recombination gametes.

These limitations in the measurement of p and in the estimation of the sampling variance of this quantity suggest the transformation, $x = p(1-p)$, for use with F_2 and F_3 interchange data. In this type of data x can be measured directly and an estimation of the sampling variance of x can be made for all recombination values. Thus, in the critical range where p approaches .5 and where little if any information concerning the identity of the recombination and non-recombination gametes is available, information does exist concerning the product of these two frequencies. This information based on x is available and can be used in adapting workable formulas for the combination of data from various sources by the method of scoring developed by FISHER (1946) and adapted for plant material by KRAMER and BURNHAM (1947).

Maximum Likelihood Formulas in Terms of x

With the substitution, $x = p(1-p)$, the expected phenotypic frequencies and the genotypic frequencies as determined by F_3 progeny rows can be expressed in terms of x. These expected frequencies are given in table 2 together with the observed numbers expressed as the quantities a to h. By the application of the method of maximum likelihood to these frequencies, formulas can be de-

TABLE 2

F_2 phenotypic and genotypic frequencies with the corresponding observed numbers in terms of x where $x=p(1-p)$.

CLASSIFICATION	F_2 PHENOTYPES			F_2 GENOTYPES IN THE A—CLASS		
	A—	*aa*	TOTAL	*AA*	*Aa*	TOTAL
Semisterile						
Expected frequencies	(1−x)/2	x/2	1/2	x/2	(1−2x)/2	(1−x)/2
Observed numbers	a	c		e	f	
Normal						
Expected frequencies	(1+2x)/4	(1−2x)/4	1/2	(1−2x)/4	x	(1+2x)/4
Observed numbers	b	d		g	h	
Total	3/4	1/4	1	1/4	1/2	3/4

veloped for the determination of linkage in any type of F_2 or F_3 interchange data. The basic concepts of this method of estimation have been reviewed and illustrated by MATHER (1946, pp. 203–208). The maximum likelihood formulas adapted for various types of F_2 and F_3 data are presented in column 2 of table 3.

TABLE 3

Maximum likelihood formulas for determination of x where $x=p(1-p)$ and formulas for the amount of information based on an estimate of x.

SOURCE OF DATA	MAXIMUM LIKELIHOOD FORMULAS	AMOUNT OF INFORMATION (i_x) PER INDIVIDUAL OR F_3 LINE
F_2, four classes	$-\frac{a}{1-x}+\frac{2b}{1+2x}+\frac{c}{x}-\frac{2d}{1-2x}$	$\frac{1}{2x(1-x)}+\frac{2}{1-4x^2}$
F_2, two classes due to recessive lethal	$-\frac{a}{1-x}+\frac{2b}{1+2x}$	$\frac{2}{(1-x)(1+2x)}$
F_2, six classes due to incomplete dominance	$\frac{c+e+h}{x}-\frac{2(d+f+g)}{1-2x}$	$\frac{2}{x(1-2x)}$
F_3, from semisterile dominant F_2's	$\frac{e}{x}-\frac{2f}{1-2x}+\frac{e+f}{1-x}$	$\frac{1}{(1-x)^2(x)(1-2x)}$
F_3, from normal dominant F_2's	$2\left(-\frac{g}{1-2x}+\frac{h}{2x}-\frac{g+h}{1+2x}\right)$	$\frac{4}{(1+2x)^2(x)(1-2x)}$

By setting the appropriate maximum likelihood formula equal to zero, the value of x which best fits the observed data is obtained, and this value of x would be the maximum likelihood estimate for the respective data. However, for any other value of x, the expression is not zero; it has a value which FISHER terms a score. This score becomes the basis for the combining of genetic data and for the estimating of linkage intensities by the method of scoring. Thus,

the maximum likelihood formulas in table 3 become the formulas for the scores (c_x) applicable to the estimation of x.

The Amount of Information

The total amount of information concerning linkage which is available in a body of genetic data depends upon the size of the sample N and an intrinsic portion i. This portion i, which depends on the value of the estimate, is referred to as the amount of information contributed by each individual or, in the case of F_3 data, by each row to the estimate of the parameter. Since x is the linkage function being estimated, the amount of information i_x may be determined by

$$i_x = S \frac{1}{m}\left(\frac{dm}{dx}\right)^2 \qquad (A)$$

where m is the expected proportion of the total in a class, dm/dx is the derivative of m with respect to x and S denotes summation over all classes (FISHER 1938, pp. 25–34). The general formulas for the determination of i_x are presented in column 3 of table 3. These values when multiplied by N, the number of individuals classified, give the total amount of information I_x furnished by each body of data.

Combining Data from Different Sources

The steps in analyzing linkage data from different sources include (1) testing the hypothesis of independent assortment between the characters being studied, (2) determining the homogeneity of various sources of data, and (3) if linkage is present, calculating a recombination value and its standard error which best fits all available data. FISHER (1946) presented the method of scoring for analyzing different sources of genetic data. Based on the transformation $x = p(1-p)$, formulas have been adapted for the method of scoring segregating data involving interchanges.

For testing independence between a factor pair and the interchange break, a convenient expression for χ^2 is given by

$$\chi^2 = \frac{c_x^2}{I_x} \qquad (B)$$

with one degree of freedom. The score c_x and the total amount of information I_x are both computed for the value of $x = .25$. The χ^2 test for homogeneity where there are n sources of data to be pooled is

$$\chi^2 = S\left(\frac{c_x^2}{I_x}\right) - \frac{(Sc_x)^2}{SI_x} \qquad (C)$$

with $n-1$ degrees of freedom and with summation over the n sources of data. An estimate of a combined x value for all data is

$$x = x' + \frac{Sc_x}{SI_x} \qquad (D)$$

where x′ represents the selected value for x and where the scores and the information again are summed over the n sources of data. Since these three basic equations are first considered for x=.25 for independence (the value of x taken for p=.5), the maximum likelihood formulas and the formulas for the amount of information i_x from table 3 are expressed for the constant x=.25 and are presented in columns 2 and 3, respectively, of table 4. The formulas in this table become the bases for the preliminary steps in the analysis of such genetic data.

TABLE 4

Formulas for the scores and for the amount of information when x=.25 for application to χ^2 for independence between the interchange break and a factor pair.

SOURCE OF DATA	FORMULAS FOR SCORES (c_x) at x=.25	INFORMATION PER F_2 PLANT OR F_3 LINE (i_x) AT x=.25
F_2, four classes	$\frac{4}{3}(b+3c-a-3d)$	$\frac{16}{3}$
F_2, two classes due to recessive letha	$\frac{4}{3}(b-a)$	$\frac{16}{9}$
F_2, six classes due to incomplete dominance	$4(c+e+h-d-f-g)$	16
F_3, from semisterile dominant F_2's	$\frac{8}{3}(2e-f)$	$\frac{128}{9}$
F_3, from normal dominant F_2's	$\frac{8}{3}(h-2g)$	$\frac{128}{9}$

The first estimate of an average x value is generally not sufficiently close to the true combined x, but it may be substituted into the original maximum likelihood formulas in table 3 to obtain a second series of scores and information. These values would be substituted into the preceding equations to obtain a second estimate of x. The procedure is repeated until the estimated average value becomes constant.

The relationships presented have been proved by FISHER for ordinary linkage experiments. These relationships also will hold for the x transformation as adapted for interchange data. Further, after these three basic equations for the method of scoring are examined, the necessity of the x transformation for a workable method of scoring becomes apparent. It has been noted that no information exists concerning the estimate of the recombination value at p=.5. Since i_p is zero for this critical value of p, I_p also would be zero and the method of scoring based on an estimate of p can not be used. The condition is obviated by the use of x, the information i_x based on x being real and finite for this range of p (table 4).

Conversion to Recombination Values

After the appropriate x values have been obtained, they may be converted readily to the recombination value p. Since $x=p(1-p)$,

$$p = \frac{1 - \sqrt{1 - 4x}}{2} . \qquad (E)$$

Also, since

$$i_p = S\frac{1}{m}\left(\frac{dm}{dp}\cdot\frac{dx}{dx}\right)^2 = S\frac{1}{m}\left(\frac{dm}{dx}\right)^2\left(\frac{dx}{dp}\right)^2 = i_x\left(\frac{dx}{dp}\right)^2$$

and

$$i_p = i_x\left[\frac{d[p(1-p)]}{dp}\right]^2 = i_x[1 - 4p(1-p)] = i_x(1 - 4x),$$

the standard error of p then becomes

$$S.E._p = \sqrt{\frac{1}{I_x(1-4x)}} . \qquad (F)$$

When x is small, the standard error of p is not greatly different from the standard error of x, but as x approaches .25 the $S.E._p$ approaches infinity.

THE DETERMNIATION OF LINKAGE INTENSITIES

HANSON and KRAMER (1949) have presented the genetic analysis of two translocations in barley from F_2 data. In one of these interchanges (Accession 301), the chromosome carrying linkage group IV was shown to be involved. The other chromosome of the interchange complex could not be established with any degree of certainty; however, the VI linkage group was thought to be involved. The factor pairs hooded vs. awned (*K*,*k*) and normal vs. xantha seedlings (*Xc*,*xc*) in linkage groups IV and VI, respectively, (ROBERTSON WIEBE, and SHANDS 1947) were used to study linkage relationships.

For additional data in the F_3, dominant F_2 plants were randomly selected in the normal and semisterile classes, and F_3 progeny rows were grown from each selected plant. Classification of these rows established the genotypes of the F_2 plants. The F_2 data from HANSON and KRAMER (1949) and the additional F_3

TABLE 5

Linkage data in the F_2 and F_3 generations involving the interchange point in accession 301 and the factor pairs K,k and Xc,xc.

		INTERCHANGE 301 VS. *K,k*			INTERCHANGE 301 VS. *Xc,xc*	
F_2		*K−*	*kk*		*Xc−*	*xcxc*
	SS	a=207	c=23	SS	a=105	lethal
	N	b=140	d=87	N	b=89	lethal
F_3		*KK*	*Kk*		*XcXc*	*Xcxc*
	SS	e=13	f=138	SS	e= 4	f=101
	N	g=57	h= 36	N	g=65	h= 24

data are presented in table 5. The letters given in this table correspond to the appropriate observed classes in the formulas of tables 3 and 4.

The complete analysis for linkage between the interchange point and *k* is illustrated in table 6. The first step in the analysis of such data would be to determine whether the interchange break and the factor pair were independently inherited. When the observed values for a, b, c, etc., in table 5 are substituted into the appropriate formulas for the score in column 2 of table 4, the scores taken at x=.25 (table 6, column 2) are obtained. The I_x values appearing in column 3 of table 6 are obtained by multiplying the fractions for the amount of information i_x in column 3 of table 4 by the number classified for each source of data. These values are the total amount of information contributed by each class when x is taken at .25 for independence. The χ^2 values for independence (Expression B) correspond to the linkage component in a

TABLE 6

The calculation of linkage between the interchange point and the K,k locus.

SOURCE OF DATA	x=.25			x=.11		
	SCORE (c_x)	I_x	$\chi^2=\frac{c_x^2}{I_x}$	SCORE	I_x	χ^2
F_2 (Four classes)	−345.3	2437	48.93	−17.1	3294	.09
F_3 (SSA)	−298.7	2148	41.54	−66.0	2222	1.96
F_3 (NA)	−208.0	1323	32.70	+28.6	2913	.28
Sum χ^2			123.17			2.33
Total	−852.0	5908	122.87	−54.5	8429	.35
Homogeneity χ^2			.30			1.98
Estimate of x			.11			.104
Recombination value p						.12±.014

partitioned χ^2 for goodness of fit. Since the three values are very highly significant, the hypothesis of independence must be abandoned. In the absence of significant χ^2 values, the analysis is complete at this point. Since linkage exists in these data, the next step is to test for population homogeneity. From relationship C, the χ^2 for population homogeneity with 2 degrees of freedom becomes 123.17−122.87=.30, where the value 122.87 is obtained by computing a χ^2 for the totals of the c_x and I_x columns. With non-significance for this test, the data from the three sources are considered homogeneous and are combined. A provisional estimate of x is obtained by applying formula D. Thus .25−852.0/5908=.11. The value .11 may now be substituted for x in the formulas in columns 2 and 3 of table 3 and the procedure repeated with this revised x value. The second estimate for x yields .104 and a third estimate, which may be calculated as a check if desired, shows no significant improvement. Applying formula E,

$$p = \frac{1 - \sqrt{1 - 4(.104)}}{2} = .12.$$

The standard error of p may be approximated from formula F,

$$S.E._p{}^* = \sqrt{\frac{1}{8429(1 - 4\times.104)}} = .014.$$

The value of $p = .12 \pm .014$ is the best estimate of the recombination value which can be obtained from the combined data.

The analysis of the data involving the interchange break and the lethal factor *xc* is made in an identical manner and is presented in table 7. Based on the highly significant χ^2 tests for independence in the F_3 data, the hypothesis of independence between the xantha locus and the interchange point must be

TABLE 7

The calculation of linkage between the interchange point and the Xc,xc locus.

SOURCE OF DATA	x = .25			x = .07		
	SCORE (c_x)	I_x	$\chi^2 = \frac{c_x^2}{I_x}$	SCORE	I_x	χ^2
F_2 (Two classes)	− 21.3	345	1.32	+43.2	366	5.10
F_3 (SSA)	−248.0	1493	41.19	−64.9	2017	2.09
F_3 (NA)	−282.7	1266	63.13	+35.4	4550	.28
Sum χ^2			105.64			7.47
Total	−552.0	3104	98.16	+13.7	6933	.27
Homogeneity χ^2			7.48			7.20
Estimate of x			.07			.072
Recombination value p						.08 ± .014

abandoned, identifying the VI linkage group with the interchange complex. Evidence of heterogeneity between the different sources of data is apparent from the χ^2 value of 7.48 for homogeneity with 2 degrees of freedom which is significant at the 5 percent level. An inspection of the individual χ^2 values show that the F_2 data are out of line. This heterogeneity could be due to misclassification of semisterility on some of the F_2 plants since the two sources of F_3 data can be shown to be homogeneous.

It is of special interest to note that the information furnished by the two classes in the F_2 is very small compared to the amount of information furnished by the F_3 progeny lines. Because of this, the F_2 data will have little effect on the final recombination value and are included for illustration. The recombi-

* For illustration the value for I_x of 8429 is taken from table 5, where x = .11. A more accurate estimate would be made with I_x taken for x = .104, but this would not change the value of the $S.E._p$ appreciably.

nation value in this case is calculated to be $.08 \pm .014$. Thus, the use of seedling lethals in studies of this type is not precluded if the tests are carried into the F_3.

DISCUSSION

The study of linkage between a translocation involving two non-homologous chromosomes and the locus of a factor pair by the use of segregating data has been outlined. It has been demonstrated that the use of F_2 and F_3 data to obtain the linkage values between the break and a gene is feasible. Through the use of the x transformation and the method of scoring, the computations are reasonably simple. A method for the determination of linkage intensities which employs the F_3 as well as the F_2 is valuable because of the additional information available; the use of seedling lethal characters in studies of this kind is not precluded because of the lack of information supplied by the F_2. The method as used here is, of course, equally applicable when only a single source of data is available. If the F_2 data are handled in this manner, F_3 data may be added without the necessity of recalculating the F_2.

One of the major difficulties in the use of the method is one which is intrinsic in the data. As a result of the failure to separate genetically recombination and non-recombination gametes, p can not be determined directly by the applica-

TABLE 8

F_2 and backcross populations which on the average will give significant deviations from independence at the 5 percent level at given recombination percentages.

RECOMBINATION	SIZE OF POPULATION		RECOMBINATION	SIZE OF POPULATION	
PERCENTAGE	BACKCROSS	F_2	PERCENTAGE	BACKCROSS	F_2
0	4	12	25	15	184
5	5	18	30	24	450
10	6	28	35	43	1,420
15	8	48	40	96	7,200
20	11	89	45	384	115,200

tion of the maximum likelihood formulas. Hence, recombination values can be inferred only indirectly, the one chosen being that which is less than .5. That such an inference is correct, however, is easily demonstrated empirically for a single source of data by calculating expected phenotypic proportions using the inferred recombination value.

A second major difficulty is the loss of information suffered because of the limitations in classification. In table 8 the population sizes necessary on the average to detect a given recombination value at the 5 percent level using backcross and 4-class F_2 data are compared, ignoring the correction for continuity. In the range of 0 to 30 percent recombination the backcross furnishes from 3 to almost 20 times the information provided by the F_2. Above 30 percent recombination, the size of populations necessary to detect linkage with F_2 data rapidly becomes prohibitive. The use of F_3 data partially offsets this difficulty. At about 30 percent recombination the F_3 classes furnish 2 to 3 times the information obtainable from the F_2. The difficulty is also partially elimi-

nated in species which have primarily alternate disjunction of the ring of 4 at metaphase I of meiosis, since in these species, as HANSON and KRAMER (1949) have pointed out, recombination should be reduced in the arms of the synaptic complex carrying the centromere, the reduction depending on the length of interstitial region involved.

Where the use of backcrosses is feasible, such data are to be preferred to F_2 data; hence, situations in which it is desired to combine the two types of data probably will be rare. Since the backcross technique permits the identification of recombination gametes as in contradistinction to the use of F_2 and F_3 material, data from the two sources are not amenable to combining by the scoring method used here. If necessary, however, they can be combined by weighting the appropriate amounts of information furnished by each source.

SUMMARY

In determining linkage values from F_2 and F_3 data involving the point of interchange between non-homologous chromosomes and a factor pair, recombination and non-recombination gametes can not be identified by phenotypic classification, and coupling and repulsion phases can not be detected. Therefore, it is essential that linkage be studied as a function of both recombination and non-recombination gametes.

Using the quantity $x=p(1-p)$, formulas were derived which permit the necessary tests for independence and homogeneity and which facilitate the combining of data and the computing of linkage from F_2 and F_3 sources of genetic material. The method was illustrated in detail using a translocation in barley.

When some of the F_2 classes are eliminated because of seedling lethality, very little information is obtained in the F_2. This difficulty can be completely obviated when the studies are carried through the F_3 generation, permitting the use of seedling lethals as marker genes. It was possible to show that the chromosome carrying linkage group VI was involved in the interchange by using the seedling lethal conditioned by *Xc,xc* as a genetic marker.

Previous data which had established linkage group IV with the interchange complex were substantiated by F_3 data.

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