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A simple method for estimating recombination percentages and linkage intensities from F_2 data: examples from *Triticum monococcum* and other self-fertilizing diploid plant species

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Summary. We offer an alternative approach to the extensively used maximum likelihood and product methods for calculating recombination values and linkage intensities from F_2 data. This new method which we designate as the square root approach is simpler than the ones in current use in that it obviates the need for formulae and tables. It can be applied to autosomal F_2 data from F_1's heterozygous in both the coupling and repulsion phases. It has greater applicability than the product method in that it can be used in all cases involving 2-, 3-, 4-, 6- and 9-class segregations regardless whether gene interaction occurs or not, provided the double recessive and other specific phenotypes are each determined by one particular genotype. The proposed method is based on the same well established genetic facts as the other two approaches. Percent recombinant gametes and therefore percent recombination are calculated by deriving the square root of the proportion of the F_2 population that expresses the double recessive or equivalent phenotype. The recombination values obtained by our method are compared with those derived by product method for 17 crosses in 7 different species and were found to be insignificantly different from the latter. The advantages and disadvantages of the square root method compared with the two most used ones are discussed.

Key words: F_2 data – Recombination values – Linkage – Square root method – Coupling – Repulsion – Self-fertilizer– *Triticum monococcum*

Introduction

The first method for the measurement of linkage intensities was presented by Bateson and Punnett in 1911. Since then various approaches have been proposed and utilized in the measurement of genetic distances between genes. In haploid eukaryotes, depending upon whether the products of each meiocyte in a given species are arranged in a linear or non-linear fashion, ordered and unordered tetrads respectively are analyzed to determine recombination values and linkage intensities (Ebersole 1956; Lindegren 1933, 1936; Papazian 1952; Pascher 1916; Perkins 1953). In cross-fertilizing species of diploid and polyploid eukaryotes, and where possible in self-fertilizing ones, testcross data are used for linkage analysis (Bateson et al. 1905; Morgan 1911a, 1911b; Punnett 1917). These two approaches provide direct information on the genetic kinds and proportions of F_1 gametes produced by dihybrids and therefore on recombination values. In self-fertilizing species however, it is often impossible and/or impractical to obtain testcross data. In such situations F_2 (and even F_3) results must be relied on to determine recombination values and linkage distances.

Several methods for calculating recombination and linkage values from F_2 data have been proposed and used (Bateson et al. 1905; Bateson and Punnett 1911; Collins 1912; Emerson 1916; Fisher 1928; Fisher and Balmukand 1928; Haldane 1919; Oweven 1928; Wellensiek 1927). However, only the maximum likelihood (Haldane 1919) and product (Fisher and Balmukand 1928) methods have been found to be efficient and are widely used. Allard (1956), Immer (1930), Immer and Henderson (1943) and Stephens (1939) have provided formulae and tables to facilitate the calculation of recombination values, using these latter two approaches.

We offer yet another approach to calculating recombination values and linkage distances from F_2 results. The method proposed is simpler than the ones in current use in that it circumvents the need for formulae and tables. In addition it is useful in many cases where gene interaction is involved as well as in all cases where the different pairs of alleles determine the expression of different pairs of traits.

The square root method for estimating linkage intensities from F_2 data: theory and method

1 Theory

The proposed method is based on well established genetic facts:

(a) Gametes unite at random.
(b) The likelihood of occurrence of a given F_2 genotype is the product of the probabilities of occurrence of the combining F_1 male and female gametes.
(c) If two pairs of alleles determine the expression of different pairs of traits, regardless of the specific allelic relationship at each locus there is only one F_2 phenotype, the double recessive one, that is always specified by one particular F_2 genotype, the one homozygous for recessive alleles at both loci.
(d) Unless there is evidence to the contrary, the frequency of crossing over is the same in both male and female meiocytes of the species under investigation.
(e) A single reciprocal exchange (crossing over) event in dihybrids between two pairs of loci in a meiocyte results in two complementary recombinant type gametes.

2 Method

If all assumptions are correct, to determine percent recombination simply derive the square root of the proportion of the F_2 population that expresses the double recessive phenotype.

(a) If the cross is in coupling – this derived value (which is designated x), e.g., 0.40 represents the proportion of F_1 male and female gametes with the parental genotype carrying recessive alleles at both loci. The proportion of the F_1 male and female gametes with the corresponding parental genotype carrying dominant alleles at both loci will have the same frequency (0.40). The recombinant gametes would constitute the remaining fraction of F_1 gametes, e.g., $1-2x=0.20$, and since both complementary recombinant genotypes are products of the same reciprocal exchange event they will occur in equal proportions, e.g., 0.10 and 0.10. This derived fraction of gametes indicates percent recombination and represents the genetic distance between linked genes. For example, if the F_1 dihybrids *BP/bp* produced parental *BP* and *bp* and recombinant *Bp* and *bP* gametes in a ratio of 0.4:0.4:0.1:0.1, percent recombination and map distance is 0.20 (20%).

Cross in Coupling

P_1 *Black pubescent* $(\frac{BP}{BP})$ × *White glabrous* $(\frac{bp}{bp})$

Gametes (BP) (bp)

F_1 *Black pubescent* $(\frac{BP}{bp})$

$F_1 \times F_1$ $\frac{BP}{bp}$ ♀ × $\frac{BP}{bp}$ ♂

Gametes (BP) (bp) (Bp) (bP) × (BP) (bp) (Bp) (bP)

Parental Recombinant Parental Recombinant

F_2	a	*Black pubescent*	134
	b	*Black glabrous*	15
	c	*White pubescent*	20
	d	*White glabrous*	31

$\frac{d}{Total} = \frac{31}{200} = 0.1550$

$x = \sqrt{0.1550} = 0.3937$ (proportion of *bp* type F_1 gametes).

Proportion of F_1 parental type gametes – *BP* (0.3937) + *bp* (0.3937) = 0.7874.

Proportion of F_1 recombinant type gametes – *Bp* (0.1073) + *bP* (0.1073) = 0.2126.

Percent recombination = $1 - 2x = 1 - 0.7874 = 0.2126 \times 100 = 21.26$.

d = number of F_2 with double recessive phenotype.
x = square root of proportion of d.

Cross in Repulsion

P_1 *Black glabrous* $(\frac{Bp}{Bp})$ × *White pubescent* $(\frac{bP}{bP})$

Gametes (Bp) (bP)

F_1 *Black pubescent* $(\frac{Bp}{bP})$

$F_1 \times F_1$ $\frac{Bp}{bP}$ ♀ × $\frac{Bp}{bP}$ ♂

Gametes (Bp) (bP) (BP) (bp) × (Bp) (bP) (BP) (bp)

Parental Recombinant Parental Recombinant

F_2	a	*Black pubescent*	101
	b	*Black glabrous*	45
	c	*White pubescent*	53
	d	*White glabrous*	2

$\frac{d}{Total} = \frac{2}{201} = 0.00995$

$x = \sqrt{0.00995} = 0.0998$ (proportion of *bp* type F_1 gametes).

Proportion of F_1 recombinant gametes – *bp* (0.0998) + *BP* (0.0998) = 0.1996.

Proportion of F_1 parental gametes – *Bp* (0.4002) + *bP* (0.4002) = 0.8004.

Percent recombination = $2x = 0.0998 \times 2 = 0.1996 \times 100 = 19.96$.

Fig. 1. Calculation of percent recombination from F_2 progenies from dihybrid F_1's in coupling and repulsion for the partially linked allele pairs *Bb* and *Pp* in *Triticum monococcum*

(b) If the cross is in repulsion the value x obtained by calculating the square root of the proportion of the F_2 population that expresses the double recessive phenotype indicates the proportion of recombinant F_1 gametes that are double recessive in both F_1 male and female meiocytes, e.g., 0.1. Of the remaining fraction (0.9) of the F_1 gametes, the corresponding double dominant F_1 gamete type would be present with the same frequency, e.g., 0.1, as the recombinant double recessive gamete, for reasons stated above. The remaining fraction (0.8) of F_1 gametes would consist of the two parental types dominant-recessive and recessive-dominant in equal frequencies. For example, if the dihybrid F_1 *Bp/bP* produced parental *Bp* and *bP* and recombinant *BP* and *bp* gametes in a ratio of 0.4:0.4:0.1:0.1, the percent recombination and map distance between the genes would be $2x = 0.2$ (20%).

3 Application of method

The calculation of percent recombination from F_2 data is illustrated in Fig. 1 which shows unpublished personal F_2 results from dihybrid F_1's in both coupling and repulsion phases for the two partially linked pairs of alleles *Bb* (*black* vs. *white* glume) and *Pp* (pubescent vs. glabrous glumes) in *Triticum monococcum* which are approximately 20 map units apart.

4 Reliability of proposed method

The reliability of our method for calculating recombination values is compared with the product approach for actual data from several authors and seventeen crosses and given in Table 1.

The product method (Fisher 1928; Fisher and Balmukand 1928) which is as efficient as the maximum likelihood one for calculating linkage intensities is relatively simple and straightforward when appropriate tables are available. The *T. monococcum* example discussed previously (Fig. 1) will be used to indicate how recombination values are calculated using this approach. Since one allele is completely dominant to the other at each of the two loci, e.g., *B* is dominant to *b* and *P* is dominant to *p* and F_2 populations from self-fertilization or intercrossing F_1's heterozygous in the same phase (coupling or repulsion) will consist of four phenotypic classes: double dominant determined by *B–P-*, dominant-recessive specified by *B–pp,* recessive-dominant given by *bbP–* and double recessive determined by *bbpp* which are designated a, b, c and d respectively. The observed frequencies for the four classes are then substituted in appropriate formulae to determine the *p* value (Allard 1956; Emerson 1916; Fisher and Balmukand 1928; Immer 1930; Owen 1928).

In repulsion *p* is the percent recombination and in coupling 1–*p* is the recombination percentage, expressed in decimal fractions. Tables have been calculated by Immer (1930); also see Allard (1956) giving the values of the ratio of the products ad/bc (for repulsion) and bc/ ad (for coupling) for different values of *p*, thus significantly reducing the labor involved in determining linkage values. The determination of linkage intensities by the product method simply resolves itself into calculating the ratio of products from F_2 data and finding the recombination percentage by interpolation in the appropriate table, a small sample of which is given in Table 1. The probable errors are obtained by dividing the probable error factor corresponding to the calculated recombination value by the square root of the number of F_2 individuals designated by N.

As an example of the use of the product method, consider the F_2 data from the *T. monococcum* F_1's heterozygous in the coupling phase (Fig. 1). The ratio of products is $bc/ad = 15 \times 20/134 \times 31 = 0.07222$. By interpolation in Table 2 we find the recombination value to be 0.195 (19.5%). From the same table, by interpolation we find that the proper factor for the probable error is 0.3039 which, divided by $\sqrt{200}$ gives a probable error of the above linkage intensity of 0.021 or 2.1% (19.5 ± 2.1).

Discussion

Unlike tetrad and testcross data, F_2 results provide indirect information on the kinds and proportions of parental and recombinant type gametes produced by dihybrid F_1's. Because analysis of F_2 results is more difficult, tedious and time consuming, whenever possible, testcross data is analyzed to determine recombination and linkage values. However, in self-fertilizing species (rarely in cross-fertilizing ones) it is often impossible and/or impractical to obtain testcross data to calculate percent recombination and F_2 (and even F_3) results must be relied on for this purpose. The most useful methods for estimating recombination values using F_2 data are the product and maximum likelihood methods. The product method is relatively simple to apply with tables such as that in Table 2. The method is limited to F_2's where four phenotypic classes occur. The method of maximum likelihood is not as easy to apply as the product one, but is quite general and may be applied to all testcross, F_2 and F_3 genetic data.

The square root method, proposed in this paper, is, in our opinion, also a satisfactory approach to calculating recombination values and linkage intensities. Moreover, unlike the product and maximum likelihood methods, it does not necessitate the use of formulae and tables. The conditions for its use are the same as those for the two currently used approaches. Like the other two methods, the proposed one can be applied to

Table 1. A comparison of recombination values derived by the proposed square root method and product method for 17 pairs of alleles in seven different self-fertilizing diploid plant species

Species	Phase of cross	Cross	F_1	F_2					Percent recombination			Source of F_2 data
									Product method		Square root method	
				a	b	c	d	Total(N)	Value	Probable error		
1. *Pisum sativum*	Coupling	*Round Yellow* × *Wrinkled Green* RR YY rr yy	*Round Yellow* Rr Yy	315	101	108	32	556	51.10	±2.2	52.02	Mendel (1866)
2. *Hordeum vulgare*	Repulsion	*Normal, male-sterile* × *Liguleless, male fertile* Li Li, ms_3 ms_3 li li, Ms_3 Ms_3	*Normal, male-fertile* Li li, Ms_3 ms_3	126	43	39	12	220	48.50	±3.5	46.71	Kasha and Walker (1960)
3. *Brassica oleracea*	Coupling	*Pubescent, dark green* × *Glossy, pale green* Hr-2 Hr-2, Pg-1 Pg-1 hr-2 hr-2, pg-1 pg-1	*Pubescent, dark green* Hr-2 hr-2, Pg-1 pg-1	279	98	91	32	500	49.98	±2.3	49.40	Sampson (1978)
4. *Lycopersicon esculentum*	Repulsion	*Narrow leaf, wiry* × *Broad leaf, normal* EE wi wi ee Wi Wi	Narrow leaf, normal Ee Wi wi	791	263	346	57	1457	40.15	±1.5	39.55	Butler (1963)
5. *Brassica oleracea*	Coupling	*Pubescent*[a] × *Pubescent*[a] Hr-2 Hr-2, Hr-1 Hr-1 hr-2 hr-2, hr-1 hr-1	*Pubescent* Hr-2 hr-2, Hr-1 hr-1	549	131	178	77	935	41.79	±1.5	42.61	Sampson (1978)
6. *Hordeum vulgare*	Repulsion	*Normal, absent*[b] × *"Uzu" present*[b] Uz Uz, als als uz uz, Als Als	*Normal, present* Uz uz, Als als	150	66	67	8	291	32.69	±3.5	33.16	Kasha and Walker (1960)
7. *Hordeum vulgare*	Coupling	*Normal, six-row* × *Male-sterile, two-row* Ms Ms VV ms ms vv	*Normal, six-row* Ms ms Vv	954	169	176	178	1477	27.85	±0.95	30.58	Kasha and Walker (1960)
8. *Hordeum vulgare*	Repulsion	*Covered, long*[c] × *Naked, short*[c] NN lb_3 lb_3 nn Lb_3 Lb_3	*Covered, short* Nn Lb_3 lb_3	643	363	319	16	1341	20.03	±1.8	21.84	Kasha and Walker (1960)
9. *Lycopersicon esculentum*	Coupling	*Normal, narrow leaf* × *Divergens, broad leaf* Di Di EE di di ee	*Normal, narrow* Di di Ee	1851	279	260	415	2805	21.79	±0.6	23.07	Butler (1963)
10. *Triticum monococcum*	Coupling	*Black, pubescent glumes* × *White, glabrous glumes* BB PP bb pp	*Black, pubescent* Bb Pp	134	15	20	31	200	19.50	±2.1	21.26	Personal unpublished data
11. *Triticum monococcum*	Repulsion	*Black, glabrous* × *White, pubescent* BB pp bb PP	*Black, pubescent* Bb Pp	101	45	53	2	201	19.60	±4.5	19.96	Personal unpublished data
12. *Triticum monococcum*	Repulsion	*Compact, awned* × *Lax, awnless* C_2 C_2, ga ga c_2 c_2, Ga Ga	*Compact, awnless* C_2 c_2, Ga ga	491	198	255	4	948	13.62	±2.1	13.0	Moseman and Smith (1954)

Species	Phase of cross	Cross	F_1	F_2					Percent recombination			Source of F_2 data
									Product method		Square root method	
				a	b	c	d	Total(N)	Value	Probable error		
13. *Lathyrus odoratus*	Coupling	*Purple, long* × *Red, short* PP LL pp ll	*Purple, long* Pp Ll	4831	390	393	1338	6952	12.00	±0.3	12.26	Bateson *et al* (1905) Punnett (1917)
14. *Hordeum vulgare*	Coupling	*Solid, normal* × *Variegated, ribbon grass* Va Va, Rb Rb va va, rb rb	*Solid, normal* Va va, Rb rb	379	9	14	123	525	4.33	±0.6	3.20	Walker *et al* (1963)
15. *Triticum monococcum*	Coupling	*Normal, green* × *Creamex, yellow* Cx Cx YY cx cx yy	*Normal, green* Cx cx Yy	1432	31	48	479	1990	3.90	±0.3	1.88	Moseman and Smith (1954)
16. *Hordeum vulgare*	Repulsion	*Green, long* × *Glossy, short* Gl_2 Gl_2, lb_2 lb_2 gl_2 gl_2 Lb_2 Lb_2	*Green, short* Gl_2 gl_2, Lb_2 lb_2	76	37	34	0	147	— (1.5)[d]	—	—	Kasha and Walker (1960)
17. *Lycopersicon esculentum*	Coupling	*Short, beaked sepal* × *Long, non-beaked sepal* Sx Sx, En En sx sx, en en	*Intermediate, beaked* Sx sx, En en	*short beaked* 59	*int beaked* 102	*long beaked* 23	*short non-beaked* 2	*int non-beaked* 22	*long non-beaked* 42	250	20.17[e]	±2.36	18.02	Bouwkamp and Honma (1971)

(a) Hr-1 and Hr-2 act additively causing pubescent leaves.
(b) Als and als cause presence vs absence of lower laterals.
(c) Lb_3 and lb_3 determine short vs long basal internodes.
(d) Percent recombination estimated from F_3 data.
(e) Percent recombination derived by maximum likelihood method.

Table 2. Product method formulae (ratio of products), their associated recombination fractions, and factors to be divided by N to obtain probable errors

Recombination fraction	Ratio of products		Factor to be divided by $\sqrt{N}$ to obtain probable error	
	ad/bc repulsion	bc/ad coupling	F_2 repulsion	F_2 coupling
0.00	0.000000	0.000000	0.000000	0.000000
0.05	0.005031	0.003629	0.6724	0.1515
0.10	0.02051	0.01586	0.6662	0.2153
0.15	0.04763	0.03915	0.6560	0.2651
0.20	0.08854	0.07671	0.6422	0.3079
0.25	0.1467	0.1328	0.6253	0.3464
0.30	0.2271	0.2132	0.6055	0.3820
0.35	0.3377	0.3259	0.5833	0.4153
0.40	0.4898	0.4821	0.5592	0.4469
0.45	0.7013	0.6985	0.5333	0.4771
0.50	1.0000	1.0000	0.5059	0.5059
0.55	1.4317	1.4260	0.4771	0.5333

autosomal F_2 data from F_1's heterozygous in both the coupling and repulsion phases. Unlike the product method which is limited to F_2 data where 4-class segregations occur, our method can be applied to all cases involving 2-, 3-, 4-, 6- and 9-class segregations regardless whether gene interaction occurs or not provided the double recessive and other specific phenotypes are each determined by one particular genotype. For example, if one allele is completely dominant to the other at both loci or if this relationship occurs at one locus and incomplete dominance of one allele over the other or codominance occurs at the second locus, the double recessive or equivalent phenotype can be used to estimate percent recombination. If incomplete dominance and/or codominance occurs at both loci then each of the 4 different phenotypes each specified by one specific homozygous genotype can be used for this purpose. This is also true for those forms of gene interaction such as dominant epistasis, duplicate dominant epistasis (duplicate genes) and cumulative complementary dominant alleles of different genes which give rise to an F_2 recessive phenotype which is always determined by one specific (double recessive) genotype.

Like the product and maximum likelihood methods, the proposed approach cannot be used to estimate recombination values when the frequency of the double recessive or equivalent phenotype is 0. In such cases one must resort to analysis of F_3 results. Obviously, the value of 0 itself is indicative of close linkage. Thus, although the square root method does not have as general an applicability as the maximum likelihood method it can be used in a greater variety of cases than the product method.

Neither the square root nor the other two methods can be used to calculate percent recombination and linkage values from F_2 data for sex (X, Z)-linked genes and autosomal genes if there is no crossing over in meiocytes of either or both sexes. For example, there is no crossing over in *Drosophila melanogaster* males (Morgan 1912, 1914). In such organisms F_2 constitutes a testcross with respect to sex-linked genes. Half of the F_2 progeny, the heterogametic individuals, can be used for direct estimation of percent recombination if the dihybrid F_1 homogametic parent is mated with a heterogametic individual that does not carry recessive alleles at both loci. The entire F_2 (both homogametic and heterogametic individuals) constitutes a testcross if the heterogametic F_1 parent carries recessive alleles at both loci. If there is no crossing over in at least one of the sexes, F_2 results from dihybrid F_1's heterozygous in both coupling and repulsion phases can be used to determine whether genes are syntenic or not and those from dihybrid F_1's in coupling can also be used to determine whether linkage exists and if so the degree thereof. This is possible because half of the F_2 constitutes a testcross progeny with respect to the genes being studied since all these individuals receive a gamete with recessive alleles at both loci from the parent in which crossing over does not occur.

For genes that are closely linked the proposed approach is more accurate in establishing percent recombination in coupling than in repulsion phase since the chance of obtaining the expected number of double recessives is greater than in repulsion. Also if the progeny size is small, the square root method may not provide as accurate a measure of amount of recombination as the other two approaches since a small deviation from the expected number of double recessives is proportionally large and can lead to a relatively large deviation from the actual percent recombination.

In conclusion, the proposed method, since it utilizes only one F_2 phenotypic class, may appear to be a less reliable method than the conventional ones which use all four F_2 phenotypic classes in estimating recombination values. Nonetheless, its applicability, dependability and accuracy, particularly in large F_2 populations, are demonstrated by the similarities of the recombination values to those using the product method as shown in Table 1. Like other methods, the one being proposed here also suffers from limitations inherent in small F_2 populations. However, for a given F_2 population, depending on the phenotypic class(es) whose value(s) deviate from expected, and the direction and extent of deviation, either the product and maximum likelihood methods or the proposed square root approach can provide a more accurate estimate of percent recombination.

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