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Estimation of preferential pairing in tetraploid × diploid hybridizations

K. G. Haynes[1,*], **W. E. Potts**[2] and **M. J. Camp**[2]

[1] U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Plant Sciences Institute, Vegetable Laboratory, Beltsville, MD 20705 USA

[2] U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Statistical Consulting and Analysis Service, Beltsville, MD 20705 USA

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Summary. Crosses made between tetraploid and diploid, 2n pollen-producing species directly transfer from one-half to the entire diploid genome from the diploid to the tetraploid level, depending on the mechanism of 2n pollen formation and the amount of crossing-over that occurs. Tetraploid plants that result from tetraploid × diploid hybridizations can be further utilized in a breeding program. It is postulated that preferential pairing between homologous chromosomes derived from the original tetraploid or diploid parent occurs in the tetraploid × diploid hybrid. Depending on the genetic divergence of the species involved, preferential pairing of homologous chromosomes may range from zero to one. Theoretical estimates of the amount of preferential pairing and the standard errors of these estimates are derived for cases where the diploid parent produces 2n gametes by either a first division or a second division restitution mechanism.

Key words: 4x – 2x hybridization – 2n gametes – Preferential pairing

Introduction

Much emphasis in recent years has been placed on interploidy and/or interspecific hybridizations in potatoes (*Solanom tuberosum* L.) (De Jong and Tai 1977; DeJong et al. 1981; Herriott et al. 1990; Mendiburu et al. 1974; Peloquin 1981, 1982), alfalfa (*Medicago sativa* L.) (Bingham 1979), *Dactylis* (Carroll and Borrill 1966; van Santen and Casler 1990), and red clover (*Trifolium pratense* L.) (Parrott et al. 1985) as a way to move valuable germ plasm from the diploid to the tetraploid level. These derived tetraploids (DT) can be further utilized in a breeding program. The diploid species have been shown to produce 2n pollen by both first and second division restitution mechanisms (Mok and Peloquin 1975). It has been thought that the breeding value of 2n pollen produced by either mechanism is related to the amount of heterozygosity the 2n pollen transmits in the cross (Mendiburu and Peloquin 1977). The frequency of 2n pollen produced under a first division restitution mechanism by an individual of genotype a_1a_2 will be: a_1a_1, $\beta/4$; a_1a_2, $1-(\beta/2)$; and a_2a_2, $\beta/4$, where β is defined as the frequency of single exchange tetrads in the diploid (Mendiburu and Peloquin 1979; Tai 1982). The frequency of 2n pollen produced under a second division restitution mechanism by this same diploid will be: a_1a_1, $(1-\beta)/2$; a_1a_2, β; and a_2a_2, $(1-\beta)/2$.

The exploitation of valuable traits from the diploid genome that are present in the DT hybrid will depend on the amount of preferential pairing that occurs between homologous chromosomes and the amount of crossing-over that occurs. In allotetraploids, the two chromosome sets derived from each parental species usually associate as bivalents, whereas in autotetraploids, they may associate as quadrivalents. In between, depending on species divergence, is a wide range of gradation known as segmental allotetraploids (Stebbins 1947). Stephens (1950) suggested that where cryptic structural differences occur between two interfertile species, the differentiation should result in preferential pairing at meiosis. Preferential pairing of homologous chromosomes has been reported in *Secale* (Elci and Sybenga 1976), induced tetraploid rainbow trout (Diter et al. 1988), and *Trifolium* (Sybenga 1988).

The purpose of this paper is to derive an estimate of preferential pairing between homologous chromosomes of DT hybrids as well as standard error of this estimate.

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[*] To whom correspondence should be addressed

Model

The diploid plant is defined as having genotype a_1a_2:a_1 represents a unique allele and a_2 represents a different unique allele. This diploid is capable of producing three kinds of gametes, depending on the mode of 2n pollen formation and the degree of crossing-over. In this paper, 2n pollen produced by a first division restitution (FDR) mechanism and a second division restitution (SDR) mechanism with no crossing-over ($\beta=0$) are considered. Non-crossover gametes have been identified (Okwuagwu and Peloquin 1981; Peloquin 1982).

The tetraploid plant is defined as having genotype $a_ia_ja_ka_m$: where a_i, a_j, a_k, and a_m are distinctly different alleles from a_1 and a_2 in the diploid. These alleles in the tetraploid need not necessarily be different from one another for the purpose of this paper; however, for this discussion, we will assume a_i, a_j, a_k, a_m represent four distinct alleles, no gametophytic selection, and chromosomal segregation ($\alpha=0$). This tetraploid will produce six different gametes in equal frequencies: a_ia_j, a_ia_k, a_ia_m, a_ja_k, a_ja_m, and a_ka_m.

First division restitution case

Assuming that the diploid a_1a_2 produces 2n gametes by a FDR mechanism, hybridization between the $a_ia_ja_ka_m$ tetraploid defined above and the diploid will produce six genotypes at the locus under consideration, each occurring with equal frequency. The resultant derived tetraploid may either be selfed or crossed with a full sib, and its progeny can be screened for the presence or absence of one or both alleles derived from the diploid.

The frequency of gametes produced by an $a_ia_ja_1a_2$ DT in the absence of preferential pairing will be: 1/6, a_ia_j; 1/6 a_ia_1; 1/6 a_ia_2; 1/6 a_ja_1; 1/6 a_ja_2; *and* 1/6 a_1a_2. Let w denote the amount of preferential pairing that occurs between homologous chromosomes derived from the same entity: $0 \le w \le 1$. If preferential pairing occurs between homologous chromosomes derived from the same entity, then the frequency of gametes produced by an $a_ia_ja_1a_2$ DT will be: $(1/3)w+1/6$, a_ia_j; $1/6-(1/6)w$, a_ia_1; $1/6-(1/6)w$, a_ia_2; $1/6-(1/6)w$, a_ja_1; $1/6-(1/6)w$, a_ja_2; and $(1/3)w+1/6$, a_1a_2. When this individual is either selfed or crossed to a full sib, $19/36+(1/9)w+(1/9)w^2$ of the progeny will express two distinct alleles derived from the contribution of the diploid, $1/36+(1/9)w+(1/9)w^2$ of the progeny will express neither allele derived from the contribution of the diploid, and $4/9-(2/9)w-(2/9)w^2$ of the progeny will express one distinct allele derived from the contribution of the diploid (Table 1). We then desire to estimate w, the amount of preferential pairing. This can be done by setting the proportion (p) of individuals that express two alleles derived from the contribution of the diploid equal to $19/36+(1/9)w+(1/9)w^2$. From this:

$$19/36+(1/9)w+(1/9)w^2-p=0. \tag{1}$$

Solving this quadratic for w under the constraint $0 \le w \le 1$ gives:

$$w=\frac{-1+3\sqrt{4p-2}}{2} \tag{2}$$

and therefore $\frac{19}{36} \le p \le \frac{3}{4}$.

Second division restitution case

Assuming that the diploid a_1a_2 produces 2n gametes by a SDR mechanism, hybridization between the $a_ia_j\ a_k\ a_m$ tetraploid defined earlier and the diploid will produce 12 genotypes at the locus under consideration, each occurring with equal frequency. The resultant derived tetraploid may either be selfed or crossed with a full sib identified as having the same allele, and its progeny can be screened for the presence or absence of that allele derived from the diploid.

The frequency of gametes produced by an $a_ia_ja_1a_1$ DT in the absence of preferential pairing will be: 1/6, a_ia_j; 1/3, a_ia_1; 1/3, a_ja_1; *and* 1/6, a_1a_1. If preferential pairing occurs between homologous chromosomes derived from the same entity, then the frequency of gametes produced

Table 1. Gametophytic genotypes, frequencies, and distribution of progeny possessing zero (0), one (1), or two (2) alleles derived from the diploid parent, which produced 2n pollen by a FDR mechanism upon selfing a derived tetraploid of genotype $a_ia_ja_1a_2$

Female gametes	Frequeny	Male gametes					
		a_ia_j $(1/3)w+1/6$	a_ia_1 $1/6-(1/6)w$	a_ia_2 $1/6-(1/6)w$	a_ja_1 $1/6-(1/6)w$	a_ja_2 $1/6-(1/6)w$	a_1a_2 $(1/3)w+1/6$
a_ia_j	$(1/3)w+1/6$	0	1	1	1	1	2
a_ia_1	$1/6-(1/6)w$	1	1	2	1	2	2
a_ia_2	$1/6-(1/6)w$	1	2	1	2	1	2
a_ja_1	$1/6-(1/6)w$	1	1	2	1	2	2
a_ja_2	$1/6-(1/6)w$	1	2	1	2	1	2
a_1a_2	$(1/3)w+1/6$	2	2	2	2	2	2

Table 2. Gametophytic genotypes, frequencies, and distribution of progeny showing zero (0) or one (1) alleles derived from the diploid parent, which produced 2n pollen by a SDR mechanism upon selfing a derived tetraploid of genotype $a_ia_ja_1a_1$

Female gametes	Frequency	Male gametes			
		a_ia_j $1/6+(1/3)w$	a_ia_1 $1/3-(1/3)w$	a_ja_1 $1/3-(1/3)w$	a_1a $1/6+(1/3)w$
a_ia_j	$1/6+(1/3)w$	0	1	1	1
a_ia_1	$1/3-(1/3)w$	1	1	1	1
a_ja_1	$1/3-(1/3)w$	1	1	1	1
a_1a_1	$1/6+(1/3)w$	1	1	1	1

by an $a_ia_ja_1a_1$ DT will be: $1/3-(1/3)w, a_ia_1$; $1/3-(1/3)w, a_ja_1$; $1/6+(1/3)w, a_ia_j$; and $1/6+(1/3)w, a_1a_1$. When this individual is either selfed or crossed to a full sib identified as having the same allele, $35/36-(1/9)w-(1/9)w^2$ of the progeny will express one distinct allele derived from the contribution of the diploid and $1/36+(1/9)w+(1/9)w^2$ of the progeny will express neither allele (Table 2). To estimate w, the amount of preferential pairing, the proportion (p) of individuals that express one allele derived from the contribution of the diploid is set equal to $35/36-(1/9)w-(1/9)w^2$. From this:

$$35/36-(1/9)w-(1/9)w^2-p=0\,. \tag{3}$$

Solving this quadratic for w under the constraint $0 \leq w \leq 1$ gives:

$$w=-\frac{1+6\sqrt{1-p}}{2} \tag{4}$$

and therefore $\frac{3}{4} \leq p \leq \frac{35}{36}$.

Estimating *W* from experimental data

Let n individual plants be scored for the presence ($X_i=1$) or absence ($X_i=0$) of both alleles (FDR) or one allele (SDR) drived from the diploid parent, depending on the mechanisms of 2n pollen formation. Given the genetic assumptions developed earlier, $X_1, \ldots, X_n$ is a random sample from a Bernoulli distribution with parameter p, i.e., the probability that $X_i=1$ is p. The uniform minimum variance unbiased estimate of p is

$$\bar{X}=\frac{1}{n}\sum_{i=1}^{n} X_i\,.$$

Let $w=w(p)$ be estimated by $\hat{w}=w(\bar{X})$. By the delta method, the asymptotic distribution of $\hat{w}$ is normal with mean w and variance

$$\left(\frac{\mathrm{d}w}{\mathrm{d}p}\right)^2 \cdot \frac{p(1\text{-}p)}{n}\,.$$

Thus, the standard error of $\hat{w}$ can be approximated by

$$\hat{\mathrm{se}}(w)=\left(\left(\frac{\mathrm{d}w}{\mathrm{d}p}|p=\bar{x}\right)^2 \cdot \frac{\bar{x}(1-\bar{x})}{n}\right)^{1/2}.$$

From this an approximate 1-α confidence interval for w is given by

$$\hat{w} \pm z_{\alpha/2} \cdot \hat{\mathrm{se}}(w).$$

First division restitution case

In the first division restitution case, the standard error of $\hat{w}$ is

$$\left(\frac{9}{4\bar{x}-2} \cdot \frac{\bar{x}(1-\bar{x})}{n}\right)^{1/2}.$$

Estimates of w and the standard errors for these estimates for various population sizes are given in Table 3.

Second division restitution case

In the second division restitution case, the standard error of w is:

$$\left(\frac{9}{4(1-\bar{x})} \cdot \frac{\bar{x}(1-\bar{x})}{n}\right)^{1/2}.$$

Estimates of w and the standard errors for these estimates for various population sizes are given in Table 4.

Chromosomal versus chromatid segregation

First division restitution case

If there is chromatid rather than chromosomal segregation, the frequency of gametes produced by an $a_ia_ja_1a_2$ DT in the absence of preferential pairing will be: 1/28, a_ia_i; 1/28, a_ja_j; 1/28, a_1a_1; 1/28, a_2a_2; 1/7, a_ia_j; 1/7, a_ia_1; 1/7, a_ia_2; 1/7, a_ja_1; 1/7, a_ja_2; and 1/7, a_1a_2. If this DT is selfed, 185/392 of the progeny will express two distinct alleles derived from the contribution of the diploid. If preferential pairing is complete between homologous chromosomes derived from the same entity, the frequency of the gametes produced by an $a_ia_ja_1a_2$ DT will be: 1/12, a_ia_i; 1/12, a_ja_j; 1/12, a_1a_1; 1/12, a_2a_2; 1/3, a_ia_j; and 1/3, a_1a_2. If this DT is selfed, 41/72 of the progeny will express two distinct alleles derived from the contribution of the diploid using the model developed earlier. Chromatid rather than chromosomal segregation will result in underestimation of the amount of preferential pairing.

Second division restitution case

If there is chromatid rather than chromosomal segregation, the frequency of gametes produced by an $a_ia_ja_1a_1$ DT in the absence of preferential pairing will be: 1/28, a_ia_i; 1/28, a_ja_j; 1/7, a_ia_j; 2/7, a_ja_1; 2/7, a_ia_1; and 3/14,

Table 3. Estimates of w, the amount of preferential pairing for observed frequencies of $\bar{x}$, the proportion of individuals possessing two distinct alleles derived from the diploid 2n pollen (FDR) parent, upon selfing a derived tetraploid, and the standard error of $\hat{w}$ for various population sizes (n)

$\bar{x}$	$\hat{w}$	n									
		100	200	300	400	500	600	700	800	900	1000
19/36	0.00	0.45	0.32	0.26	0.22	0.20	0.18	0.17	0.16	0.15	0.14
20/36	0.21	0.32	0.22	0.18	0.16	0.14	0.13	0.12	0.11	0.11	0.10
21/36	0.37	0.26	0.18	0.15	0.13	0.11	0.10	0.10	0.09	0.09	0.08
22/36	0.50	0.22	0.16	0.13	0.11	0.10	0.09	0.08	0.08	0.07	0.07
23/36	0.62	0.19	0.14	0.11	0.10	0.09	0.08	0.07	0.07	0.06	0.06
24/36	0.72	0.17	0.12	0.10	0.09	0.08	0.07	0.07	0.06	0.06	0.05
25/36	0.82	0.16	0.11	0.09	0.08	0.07	0.06	0.06	0.06	0.05	0.05
26/36	0.91	0.14	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.05	0.05
27/36	1.00	0.13	0.09	0.08	0.06	0.06	0.05	0.05	0.05	0.04	0.04

Table 4. Estimates of w, the amount of preferential pairing for observed frequencies of $\bar{x}$, the proportion of individuals possessing one distinct allele derived from the diploid 2n pollen (SDR) producing parent, upon selfing a derived tetraploid, and the standard error of $\hat{w}$ for various population sizes (n)

$\bar{x}$	$\hat{w}$	n									
		100	200	300	400	500	600	700	800	900	1000
27/36	1.00	0.13	0.09	0.08	0.06	0.06	0.05	0.05	0.05	0.04	0.04
28/36	0.91	0.13	0.09	0.08	0.07	0.06	0.05	0.05	0.05	0.04	0.04
29/36	0.82	0.13	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.04	0.04
30/36	0.72	0.13	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.05	0.04
31/36	0.62	0.14	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.05	0.04
32/36	0.50	0.14	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.05	0.04
33/36	0.37	0.14	0.10	0.08	0.07	0.06	0.06	0.05	0.05	0.05	0.05
34/36	0.21	0.15	0.10	0.08	0.07	0.07	0.06	0.06	0.05	0.05	0.05
35/36	0.00	0.15	0.10	0.09	0.07	0.07	0.06	0.06	0.05	0.05	0.05

a_1a_1. If this DT is selfed, 187/196 of the progeny will express one allele derived from the contribution of the diploid. If preferential pairing is complete between homologous chromosomes derived from the same entity, the frequency of gametes produced by an $a_ia_ja_1a_1$ DT will be: 1/12, a_ia_i; 1/12, a_ja_j; 1/3, a_ia_j; and 1/12, a_1a_1. If this DT is selfed, three-fourths of the progeny will express one allele derived from the contribution of the diploid. Chromatid rather than chromosomal segregation will result in a negligible underestimation of the amount of preferential pairing.

Discussion

Previously, preferential pairing of homologous chromosomes could be detected through the use of Chi-square tests or cytologically in some cases. Significant deviations between the number of individuals expected to show a certain trait and the number of individuals actually observed to possess that trait were indicative of preferential pairing. In this paper, actual estimates of preferential pairing and the standard errors of these estimates for matings involving DTs have been derived. With an understanding of this methodology, the reader should be able to derive estimates of preferential pairing and the standard errors of these estimates for his own situation.

Under the assumptions of the model given, chromatid rather than chromosomal segregation in the DT could substantially underestimate the amount of preferential pairing in the FDR case. With chromosomal segregation, the proportion of individuals expected to express both alleles from the contribution of the diploid ranges from 0.53 to 0.75, depending on the amount of preferential pairing. With chromatid segregation, this proportion ranges from 0.47, with no preferential pairing, to 0.57, with complete preferential pairing.

However, under the assumptions of the model given, chromatid rather than chromosomal segregation in the DT would have a negligible effect on the estimate of the amount of preferential pairing in the SDR case. This is because the proportion of individuals expected to express one allele from the contribution of the diploid is nearly the same for both chromatid and chromosomal segregation.

In principle, this method could be extended to the case with crossing-over in the DTs, if the crossing-over rate is known or could be estimated by some other method. Of course, in that case, the sampling variance of the estimate would be complicated and is beyond the scope of this paper. However, the crossing-over rate is likely to be a function of the degree of homology that exists between the homologous chromosomes of the two parental lines. For practical purposes, if the two parental lines were widely divergent to the extent that they exhibited almost complete preferential pairing, crossing-over, if it did occur, might lead to sterility or partial sterility in the next generation.

Several conclusions can be drawn from the computations derived in this paper. First, it must be possible to phenotypically differentiation DTs possessing a_1a_1, a_1a_2, or a_2a_2 from the contribution of their diploid parent. Without this, no estimate of preferential pairing is possible. Second, if 2n pollen is produced by a FDR mechanism and $\beta=0$ and there is little preferential pairing, it will be extremely difficult to obtain a precise estimate of the amount of preferential pairing with "small" population sizes. However, if preferential pairing of homologous chromosomes approaches unity, a more precise estimate of the amount of preferential pairing can be obtained with smaller population sizes. Third, if 2n pollen is produced by a SDR mechanism and $\beta=0$, there is little gain in the precision of the estimate of preferential pairing with increasing population sizes over 200–300.

The derivations in this paper are based on the assumption that the diploid parent involved in the 4x – 2x cross is not directly derived from the tetraploid parent. Haploids (2n = 2x = 24) extracted from tetraploid (2n = 4x = 48) potatoes are easy to obtain, and haploid-species hybrids in potatoes generally have normal chromosomal pairing (Hougas and Peloquin 1958; Peloquin et al. 1966). Preferential pairing is expected to range from non existent or extremely small in intraspecific hybridizations to fairly large in interspecific hybridzations. This paper derives a theoretical estimate of the amount of preferential pairing of homologous chromosomes that should be applicable to both intraspecific and interspecific 4x – 2x hybridizations.

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